TECHNICAL REPORT

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Outcome of the public consultation on the draft Scientific Opinion on the 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

European Food Safety Authority (EFSA)
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Abstract

The European Food Safety Authority (EFSA) carried out a public consultation to receive input from interested parties on the 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. This draft scientific opinion was prepared by the GMO Panel, supported by the Working Group on Molecular Characterization. The draft opinion was endorsed by the EFSA GMO Panel for public consultation on 1st April 2020. The written public consultation was open from 15 April 2020 until 5 June 2020. EFSA received comments from 51 different interested parties. EFSA and its GMO Panel wish to thank all stakeholders for their contributions to this work. 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 14 October 2020 and will be published in the EFSA Journal.

Key words: Site-directed nuclease, oligonucleotide-directed mutagenesis, transgenesis, off-target, genetically modified plants, risk assessment, EFSA guidance

Requestor: European Commission

Question number: EFSA-Q-2020-00098

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1. Introduction

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

1.1.1. Background

The judgment of the Court of Justice of the European Union in Case C-528/16[1] on mutagenesis has clarified that Directive 2001/18/EC[2] is applicable to genetically modified organisms (GMOs) obtained by mutagenesis techniques that have emerged since its adoption ('new mutagenesis techniques').

Directive 2001/18/EC regulates the deliberate release of GMOs into the environment. In 2010, the EFSA Panel on Genetically Modified Organisms issued the Guidance on the environmental risk assessment of genetically modified (GM) plants (EFSA GMO Panel 2010) and in 2011 the Guidance on the risk assessment of food and feed from GM plants (EFSA GMO Panel 2011). Following a request of the European Commission, in 2012 EFSA published a Scientific opinion addressing the safety assessment of plants developed using zinc finger nuclease 3 and other site-directed nucleases with similar function (SDN-3) (EFSA GMO Panel (2012), hereafter 'EFSA Scientific Opinion on SDN-3'). In this Scientific Opinion, the assessment methodology applied by the EFSA GMO Panel was to compare the hazards associated with plants produced by the SDN-3 technique with those obtained by conventional plant breeding techniques and by currently used transgenesis. Among the conventional plant breeding techniques, the EFSA GMO Panel considered certain mutation breeding techniques that emerged before the adoption of Directive 2001/18/EC and that are used as a tool to create genetic variation.

The Scientific Opinion concluded that 'the SDN-3 technique can minimize hazards associated with the disruption of genes and/or regulatory elements in the recipient genome. Whilst the SDN-3 technique can induce off-target changes in the genome of the recipient plant, these would be fewer than those occurring with most mutagenesis techniques. Furthermore, where such changes occur, they would be of the same types as those produced by conventional breeding techniques.'

The EFSA GMO Panel also concluded that its 2010 and 2011 guidance documents 'are applicable for the evaluation of food and feed products derived from plants developed using the SDN-3 technique and for performing an environmental risk assessment. However, on a case-by-case basis lesser amounts of event-specific data may be needed for the risk assessment of plants developed using the SDN-3 technique.'

1.1.2. Terms of Reference

Against this background, the European Commission, in accordance with Article 29 of Regulation (EC) No 178/2002, asked EFSA to address the following two terms of reference (ToR):

1. To advise whether the assessment methodology described in section 4 of the EFSA scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function may be applicable, in whole or in part, to plants developed with type 1 and type 2 site-directed nucleases and with oligonucleotide-directed mutagenesis.

If the advice to ToR1 is affirmative, the Commission would ask EFSA, in accordance with Article 29 of Regulation (EC) No 178/2002:

2. To advise whether the conclusions of the EFSA 2012 Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function are valid, in whole or in part, to plants developed with type 1 and type 2 site-directed nucleases and with oligonucleotide-directed mutagenesis.

[1] Judgment of 25 July 2018, *Confédération paysanne and Others v Premier ministre and Ministre de l'agriculture, de l'agroalimentaire et de la forêt*, C-528/16, ECLI:EU:C:2018:583

[2] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC - Commission Declaration. OJ L 106, 17.4.2001, p. 1–39.

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 was released for public consultation from 15 April 2020 until 5 June 2020 by means of an electronical comment submission tool together with explanatory text on the EFSA website (See Appendix A). Comments were received from 51 interested parties from 17 countries. Table 1 provides an overview on the interested parties that have submitted comments through the electronic submission (Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG), CropLife Canada, Plant Biotechnology Society, Cornell University's Alliance for Science, Association Française des Biotechnologies Végétales, GMWatch, FUTURAGRA, Greenpeace European Unit, Fachstelle Gentechnik und Umwelt, Società Italiana di Genetica Agraria - Italian Society of Agricultural Genetics (SIGA), GenØk-centre for biosafety, Testbiotech and Umweltbundesamt (Environment Agency Austria) uploaded additional files in the online tool). One contribution from the European Network of Scientists for Social and Environmental Responsibility (ENSSER) from Germany was submitted as pdf by email within the deadline.

Table 1: Overview on stakeholder comments received

Stakeholder	Category [a]	Country
Agriculture and Food Systems Institute (AFSI)	NGO	United States
Association Française des Biotechnologies Végétales	NGO	France
Associazione Luca Coscioni per la libertà di ricerca scientifica	NGO	Italy
Austrian Agency for Health and Food Safety (AGES)	National authority	Austria
BIOTRIN, z.s.	NGO	Czech Republic
BUND e.V. / Friends of the Earth Germany	NGO	Germany
Comisión Nacional de Bioseguridad, Ministerio para la Transición Ecológica y el Reto Demográfico (MITECO)	National authority	Spain
Cornell University's Alliance for Science (includes 22 contributors)	University/public research institute	United States
Corporate Europe Observatory	NGO	Belgium
Corteva Agriscience	Private sector (e.g. industry, consultancy, etc.)	Belgium
COST Action CA18111 - Plant genome editing – a technology with transformative potential (PlantEd)	Others	Germany
CropLife Canada	Private sector (e.g. industry, consultancy, etc.)	Canada
ENSSER The European Network of Scientists for Social and Environmental Responsibility	NGO	Germany
Environmental association Za Zemiata	NGO	Bulgaria
EuropaBio	Private sector (e.g. industry, consultancy, etc.)	United Kingdom
European Coordination Via Campesina	Private sector (e.g. industry, consultancy, etc.)	Belgium
European Plant Science Organisation, EPSO	International organization	Belgium
Euroseeds	Private sector (e.g. industry, consultancy, etc.)	Belgium
Fachstelle Gentechnik und Umwelt	Others	Germany
Federal Agency for Nature Conservation	National authority	Germany
Federal Office of Consumer Protection and Food Safety (BVL), Competent Authority according to Directive 2001/18/EC	National authority	Germany

French agency for Food, Environmental and Occupational Health & Safety (Anses)	Others	France
FUTURAGRA	Others	Italy
Ganesh kumar	on personal capacity	India
GenØk-centre for biosafety	Others	Norway
German Plant Breeders' Association (BDP - Bundesverband Deutscher Pflanzenzuechter e.V.)	Private sector (e.g. industry, consultancy, etc.)	Germany
GMO Office, National Institute of Public Health and the Environment (RIVM)	Others	Netherlands
GMWatch	NGO	United Kingdom
Greenpeace European Unit	NGO	Belgium
Haut Conseil des biotechnologies (High Council for Biotechnology)	Other	France
Institute of experimental Botany, Czech Academy of Science	University/public research institute	Czech Republic
International Seed Federation	Private sector (e.g. industry, consultancy, etc.)	Switzerland
Julius Kühn-Institut	National authority	Germany
Kleter Gijs A.	on personal capacity	Netherlands
Logos Environmental	Private sector (e.g. industry, consultancy, etc.)	United Kingdom
Ministry of Agriculture, Livestock and Food Supply of Brazil	National authority	Brazil
National Food Institute, Technical University of Denmark	University/public research institute	Denmark
Nature et Progrès Belgique	NGO	Belgium
Norwegian Scientific Committee for Food and Environment (VKM)	National authority	Norway
Plant Biotechnology Society	Others	Germany
Plantum - Netherlands seed association	Private sector (e.g. industry, consultancy, etc.)	Netherlands
Sciensano	University/public research institute	Belgium
Scientific Comittee for GM food and feed	Others	Czech Republic
Scientific Committee for GM food and Feed, Advisory Body, Czech Republic	Others	Czech Republic
SETA (Science and Technology in Agriculture)	NGO	Italy
Società Italiana di Genetica Agraria - Italian Society of Agricultural Genetics (SIGA)	Others	Italy
Testbiotech	NGO	Germany
Umweltbundesamt (Environment Agency Austria) on behalf of the Austrian lead Competent Authority, the Federal Ministry of Social Affairs, Health, Care and Consumer Protection.	National authority	Austria
Union Française des Semenciers	Private sector (e.g. industry, consultancy, etc.)	France
VIB	University/public research institute	Belgium
Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG)	NGO	Germany

[a] as specified by the commenter

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

The comments received were duly evaluated by the EFSA GMO Panel WG on Molecular Characterization on the evaluation of the 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. 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

Stakeholder	Chapter	Comment	EFSA response	Comment number
ENSSER	Abstract	It will need to reflect the alterations in the text Needs to reiterate the precautionary principle, and its role for risk assessment	The abstract has been revised to reflect the changes of the main text of the opinion.	1
Institute of experimental Botany, Czech Academy of Science	Abstract	We agree with the EFSA scientific opinion in that the guidelines for risk assessment of plants created with SDN3 techniques are not directly applicable to plants developed using SND 1 and SND 2, since such plants typically do not contain any foreign DNA. We further agree with the opinion that there are no new hazards specifically linked to the modification produced via SDN-1, SDN-2 and/or ODM when to conventional breeding.	The GMO Panel thanks for the comment.	2
Associazione Luca Coscioni per la libertà di ricerca scientifica	Abstract	Associazione Luca Coscioni (ALC) and Science for Democracy (SD) are two connected no-profit association for the promotion of scientific research. ALC (https://ec.europa.eu/transparencyregister/public/consultation/displaylobbyist.do?id=27570996265-42) promotes civil and human rights among which those related to scientific knowledge and awareness, supporting scientific research from stem cell to plant genetic improvements. SD (https://www.sciencefordemocracy.org/) says and reminds that Science is recognized and protected as a fundamental human right; it is enshrined in article 27 of the 1948 Universal Declaration of Human Rights and in article 15 of the International Covenant on Economic, Social and Cultural Rights (ICESCR). Both organizations endorse the definition expressed by the GMO EFSA Panel that genetic modification introduced via SDN-1, SDN-2 or ODM in case foreign DNA is not present in the final product, results in the correction of plant endogenous genomic sequences without the insertion of exogenous DNA. In this case, the EFSA definition is valid independently of the protocol or technology used to achieve this genomic variation, which we believe is scientifically correct. Also, we share the opinion that there are no new hazards specifically linked to the genomic modification produced via SDN-1, SDN-2 and ODM as compared to both SDN-3 and conventional breeding. We would like to point out that the term "exogenous" DNA, although currently used in the lab to define DNA inserted in a nucleus and not originally belonging to it, could be misleading: exogenous DNA could be rather identical to the nuclear DNA where it has been inserted, and even coming from the same species. We therefore suggest a modification of the term "exogenous".	The GMO Panel thanks for the comment. The use of the term "exogenous" has been re-evaluated taking into consideration all the comments received on this aspect. The GMO Panel still considers the use of this term appropriate to indicate any sequence of DNA originating outside the plant being modified which can be introduced naturally or by technological intervention. Please consider that this definition is also included in the SAM explanatory note. EUROPEAN COMMISSION (2017). New techniques in agricultural biotechnology. CEU. SAM_ADV, Directorate-General for Research and Innovation, 28 April 2017.	3
Euroseeds	Abstract	Euroseeds welcomes the opportunity to comment on the EFSA opinion to assess whether the section 4 (hazard identification) and the conclusions of the opinion on SDN-3 are valid for plants developed via SDN-1, SDN-2, and oligonucleotide-directed mutagenesis (ODM). Euroseeds agrees with the key findings of the GMO Panel which did not identify any additional hazard associated to the use of the SDN-1, SDN-2 and ODM approaches (further referred to as targeted mutagenesis approaches) as compared to both SDN-3 and conventional breeding techniques, including conventional random mutagenesis. Euroseeds agrees with the EFSA view that off-target analysis would be of "very limited value for the risk analysis". Following the initial steps to increase genetic variability including e.g. conventional random mutagenesis plant breeders use a system of crossing and selection to remove unwanted phenotypes.	The GMO Panel thanks Euroseeds for the comment.	4

SETA (Science and Technology in Agriculture)	Abstract	SETA (Science and Technology for Agriculture www.setanet.it) is a free cultural association of scientists, farmers, technicians, full professors and entrepreneurs active in the public debate to promote a science-based Agriculture. We agree with the GMO EFSA Panel that genetic modification introduced via SDN-1, SDN-2 and ODM do not add new additional risks as compared to SDN-3 or conventional breeding. 16-times in the text, and twice in the Abstract, is reported the term: "exogenous DNA". When the so-called exogenous DNA comes from the same species (and thus could be transferred by conventional breeding) or subject to a spontaneous or induced mutagenesis, it should be considered an "endogenous" DNA. The term "exogenous" should be thus modified.	The GMO Panel thanks for the comment. The use of the term "exogenous" has been re-evaluated taking into consideration all the comments received on this aspect. The GMO Panel still considers the use of this term appropriate to indicate any sequence of DNA originating outside the plant being modified which can be introduced naturally or by technological intervention. Please consider that this definition is also included in the SAM explanatory note. EUROPEAN COMMISSION (2017). New techniques in agricultural biotechnology. CEU. SAM_ADV, Directorate-General for Research and Innovation, 28 April 2017.	5
Union Française des Semenciers	Abstract	Union Française des Semenciers (UFS) agrees with the main outcomes of the GMO Panel assessment. They did not see any additional hazard associated to the use of the SDN-1, SDN-2 and ODM techniques (further referred to as targeted mutagenesis approaches) as compared to both SDN-3 and conventional breeding techniques, including traditional random mutagenesis. UFS agrees with EFSA that off-target analysis would be of "very limited value for the risk analysis". UFS reminds that, after increasing genetic variability (including via. traditional random mutagenesis), every breeding programme includes a combination of crossings, selection steps and years of observations and analyses toward the development of new improved and safe varieties by removing undesired phenotypes.	The GMO Panel thanks for the comment.	6
Scientific Comittee for GM food and feed	Abstract	In the abstract, it would be appropriate to state why the applicability of the SDN-3 risk assessment to the risk assessment for SDN-1, SDN-2 and ODM is considered. It is not clear that the specific SDN-1, SDN-2 and ODM risk assessments have not yet been approved and that there is no other reason to consider the SDN-3 safety assessment for other SDN types. We suggest to improve the rational better accordingly in the main text. It could be considered to modify the following sentence: "Furthermore, the GMO Panel considers that the existing Guidance for risk assessment of food and feed from genetically modified plants (EFSA GMO Panel, 2011) and the Guidance on the environmental risk assessment of genetically modified plants (EFSA GMO Panel, 2010) are sufficient but can be only partially applied to plants generated via SDN-1, SDN-2 and ODM." A possible more appropriate and accommodating formulation: "Furthermore, the GMO Panel considers that the existing Guidance for risk assessment of food and	The GMO Panel reviewed the abstract and considers that the original sentence correctly reflects the information provided in the main text.	7

		feed from genetically modified plants (EFSA GMO Panel, 2011) and the Guidance on the environmental risk assessment of genetically modified plants (EFSA GMO Panel, 2010) are more than sufficient so only a subset of the requirements should be partially applicable (if the ,meaning remain the same) to plants generated via SDN-1, SDN-2 and ODM."		
Scientific Committee for GM food and Feed, Advisory Body, Czech Republic	Abstract	In the abstract, it would be appropriate to state why the applicability of the SDN-3 risk assessment to the risk assessment for SDN-1, SDN-2 and ODM is considered. It is not clear that the specific SDN-1, SDN-2 and ODM risk assessments have not yet been approved and that there is no other reason to consider the SDN-3 safety assessment for other SDN types. We suggest to improve the rational better accordingly in the main text. It could be considered to modify the following sentence: "Furthermore, the GMO Panel considers that the existing Guidance for risk assessment of food and feed from genetically modified plants (EFSA GMO Panel, 2011) and the Guidance on the environmental risk assessment of genetically modified plants (EFSA GMO Panel, 2010) are sufficient but can be only partially applied to plants generated via SDN-1, SDN-2 and ODM." A possible more appropriate and accommodating formulation: "Furthermore, the GMO Panel considers that the existing Guidance for risk assessment of food and feed from genetically modified plants (EFSA GMO Panel, 2011) and the Guidance on the environmental risk assessment of genetically modified plants (EFSA GMO Panel, 2010) are more than sufficient so only a subset of the requirements should be partially applicable (if the ,meaning remain the same) to plants generated via SDN-1, SDN-2 and ODM."	The GMO Panel reviewed the abstract and considers that the original sentence correctly reflects the information provided in the main text.	8
German Plant Breeders' Association (BDP - Bundesverband Deutscher Pflanzenzuechter e.V.)	Abstract	BDP appreciates the opportunity to provide comments on the above mentioned EFSA opinion and acknowledges the efforts of EFSA to conduct a balanced and science based analysis. BDP agrees to and supports the key outcome of the GMO Panel analysis that no additional hazard associated with applying SDN-1, SDN-2 and ODM approaches to plants in comparison to both SDN-3 and conventional breeding techniques could be identified. In addition BDP fully agrees with the conclusion that any "requirements which are linked to the presence of foreign DNA are not relevant for the risk assessment of plants developed via SDN-1, SDN-2, and ODM approaches" as long as no foreign DNA is present in the final plant. Importantly EFSA also states that "because off-target effects in SDN- and ODM-based approaches is negligible compared to conventional plant breeding, the GMO Panel considers that the analysis of potential off-targets would be of very limited value for the risk analysis" (line 352-354) – a conclusion that BDP fully supports. More than that, during the breeding process breeders apply crossing and selection systematically to remove any unwanted phenotypes and detrimental effects.	The GMO Panel thanks BDP for the comment.	9
Plantum - Netherlands seed association	Abstract	Plantum is pleased with the main findings, that a) no hazards additional to conventional breeding, including random mutagenesis are identified, and b) "analysis of off-targets would be of very limited value for risk analysis". The logical next conclusion was however not presented, namely that because no additional hazards have been identified, risk assessments additional to those applied in conventional breeding would not be needed. We assume that this is so because that question was not part of the ToR.	The GMO Panel thanks for the comment. The scientific opinion has been developed by strictly adhering to the terms of reference (ToR) provided by the European Commission. Indeed, the conclusions summarize the	10

			findings within the boundaries of the ToR.	
COST Action CA18111 - Plant genome editing – a technology with transformative potential (PlantEd)	Abstract	PlantEd agrees with and wishes to emphasize the main finding of the EFSA Panel on Genetically Modified Organisms which could not find any new hazards specifically linked to the genomic modification produced via SDN-1, SDN-2 and ODM as compared to both SDN-3 and conventional breeding. Applying the precautionary principle as developed in the Commission's Communication (COM/2000/0001) and in European Court's case law and EU legislation, we trust that this is an important indication for the evaluation that the Commission is conducting in response to the Council's requests (Council Decision (EU) 2019/1904). Furthermore, PlantEd also agrees that the considerations relating to the introduction of a transgene, in the existing guidance for risk assessment of food and feed from genetically modified plants and the guidance on the environmental risk assessment of genetically modified plants, are not relevant for the risk assessment of plants developed via SDN-1, SDN-2, and ODM approaches in case the genome of the final product does not contain exogenous DNA.	The GMO Panel thanks for the comment.	11
French agency for Food, Environmental and Occupational Health & Safety (Anses)	Abstract	Page 0, line 22: "foreign DNA": the term "exogenous DNA", which is defined in the glossary, should be preferred. Page 0, line 23: "Overall, the GMO Panel did not identify new hazards…": this is true as long as the absence of effectors (DNA, RNA, protein) has been demonstrated. Page 0, lines 25-27: "the GMO Panel considers that the existing Guidance for risk assessment of food and feed from genetically modified plants (EFSA GMO Panel, 2011) and the Guidance on the environmental risk assessment of genetically modified plants (EFSA GMO Panel, 2010) are sufficient": this is not true for the analysis of potential off-target effects. Page 0, line 29: "foreign DNA": the term "exogenous DNA", which is defined in the glossary, should be preferred.	The GMO Panel thanks for the comment. Regarding comment to lines 22 and 29, the text has been amended accordingly. Regarding comment to lines 23 and 25-27, the GMO Panel refers the contributor to the refers the contributor to the related comments and the responses provided (section 3.2 of the opinion).	12
Corteva Agriscience	Abstract	As Corteva Agriscience, we are committed to innovation and strongly feel that innovative technologies, such as CRISPR genome editing, can help us grow healthy and nutritious food that is better for people and the environment. However, regulatory barriers can significantly limit its application in agriculture and benefits to the society at large. Please find more about our commitment at crispr.corteva.com. Considering the importance of the topic we would ask that EFSA GMO Panel widens its view when developing an answer for this relatively narrowly phrased question from the European Commission. Therefore, we ask EFSA to give the principle of proportionality a more prominent place in the evaluation of this mandate. The principle of proportionality is set out in Article 5 of the EU Treaty (TEU) , and has been included in the general food law which states "In accordance with the principle of proportionality as set out in Article 5 of the Treaty, this Regulation does not go beyond what is necessary in order to achieve the objectives pursued" (Regulation (EC) No 178/2002) and thus it is a principle that needs to be kept in mind in the evaluation of every mandate. In accordance with the principle of proportionality, EFSA would be expected to ensure that its measures and requests are appropriate to achieve the overall objective of safety, and do not go beyond what is necessary to achieve that goal. It is not proportionate that in case of two almost identical plants with similar risk profiles one product would have more regulatory requirements merely because of the method used for its development. Therefore, to create an equal playing field, we ask that EFSA makes clearer	The GMO Panel thanks for the comment. The scientific opinion has been developed by strictly adhering to the terms of reference (ToR) provided by the European Commission. Indeed, the conclusions summarize the findings within the boundaries of the ToR.	13

		which requirements developed for transgenic plants (or novel foods) are necessary and proportionate to protect the European consumer, animals and the environment to the same level as would occur for conventional bred crops. As described by many risk assessments bodies globally a molecular characterization of the gene edited plant (to confirm absence of unwanted transgenic sequences), which allows to populate the problem, should in many cases be sufficient to reach the same level of protection as for conventionally bred plants. Lines 23-25: In light of the conclusion that no new hazards are "specifically linked to the genomic modifications produced via SDN-1, SDN-2 and ODM as compared to both SDN-3 and conventional breeding" (in bold by Corteva), we ask EFSA to confirm and additionally clarify that the purported "hazards" are no different than those from plants developed using techniques with a long history of safe use. We also ask to include into the Abstract two specific conclusions made by EFSA and reflected in the text: 1. About the off-targets mutations in comparison to conventional breeding and their very limited value for the risk analysis. This conclusion is very important to highlight given how much intentional and unintentional misinformation is being circulated on this topic, including the popular press that, in particular, affects public opinion on the technology. 2. Regarding the scope of this opinion being also applicable to base editing and prime editing (comment to Section 3.1.2, lines 241-243).		
Haut Conseil des biotechnologies (High Council for Biotechnology)	Abstract	l. 19-20. "without the insertion of exogenous DNA": Is the difference between the molecular mechanisms involved in SDN-2 and SDN-3 so clear-cut with respect to the notion of insertion of exogenous DNA? l. 22. "in case foreign DNA is not present", and l. 31: "in case the genome of the final product does not contain exogenous DNA": This may be confusing and should be made clearer. l. 27-28. "are sufficient but can be only partially applied": unclear. Consistent with our analysis, we suggest: "are partly applicable" l. 28-31. Consistent with our analysis, we suggest the following alternative: "Depending on the cases, requirements related to the insertion of transgenes may not be needed while others may be added regarding assessment of the absence of any transgene or any DNA sequence potentially derived from the methods used to generate the intended modification, assessment of off-target effects, further analysis in case an active SDN module is still present in the final product, and specific consideration regarding multiplexing where relevant." The abstract should also stress that the amount of experimental data needed for the risk assessment will vary more according to the novelty of the trait than according to the type of techniques involved.	Regarding comment to line 19-20, the operational definition of SDN-2 used in the opinion implies that the template DNA deployed with this approach does not integrate into the genome. Should any exogenous DNA integrate into the genome, all the considerations described in this opinion on this occurrence apply. Regarding comment to line 22, the term "exogenous" has been revised and used consistently throughout the opinion. Regarding comment to lines 27-28, the GMO Panel has revised the text of the opinion based on the comments received. The GMO Panel considers the wording still appropriate.	14

			Regarding comment to lines 28-31, the GMO Panel considers the EFSA guidance still sufficient and on a case-by-case bases only a subset of the data would be needed.	
Testbiotech	Abstract	[line 23 after first bullet, up to line 31: Delete and replace text:] "However, even if no additional DNA is inserted, the specific pattern of intended and unintended genetic changes caused by SDN-1 and SDN-2 applications needs to be assessed case by case. SDN applications have to be regarded as biological mutagens that, unlike chemical or physical mutagens, can directly and specifically interact with the biological mechanisms in the cell on the level of the genome and/or epigenome. These applications have a high potential to penetrate the genome and generate profound alterations in the biological characteristics of plants without introducing any additional DNA sequences. These changes will typically give rise to biological characteristics, such as changes in plant composition that exceed the range of characteristics achieved by previous plant breeding methods. The risks associated with the release, cultivation and consumption of these plants need to be fully investigated before any conclusions on the safety of the new organisms can be drawn. In addition, there is a wide range of specific on-target and off-target effects of SDN-1 and SDN-2 interventions, largely depending on various parameters of the specific technical processes such as: (i) the specific nuclease(s) used; (ii) the target organism and its tissue, respectively; (iii) the targeted gene(s); (iv) the way in which the components are introduced into the cells; (v) the dosage of the nuclease(s); (vi) if CRISPR/Cas is applied, the guide RNA used and (vii) duration of the intervention. All these technical details determine the precision as well as the efficiency of an intervention and also have to be taken into account during risk assessment. The methodology and guidance for risk assessment needs to be adopted accordingly, taking into account the restrictions associated with the comparative approach and complex challenges posed by SDN-1 and SDN-2 plants in environmental risk assessment. The requirements for a more detailed risk assessment are not solely linked to the presence of foreign DNA, but are relevant for all genome-edited plants, including those that were developed with SDN-1, SDN-2 and whether the genome of the final product does not contain any exogenous DNA. Since these issues are not, or only partially, addressed in the previous EFSA opinion on SDN-3 (EFSA 2012a), the methodology and conclusions derived in this earlier document are not sufficient to guide risk assessment of SDN-1 and SDN-2 applications. As far as ODM is concerned, the EFSA panel could not reach final conclusions, since there is a substantial lack of data on the side effects associated with the specific method. Whatever the case, detailed examination of an organism's genetic and overall biological characteristics, starting with the process that was used to introduce changes in the genome of the organism, is needed to decide whether the organism is safe. The set of data needed for risk assessment will be dependent on each case and cannot generally be limited by criteria such as the insertion of additional genes."	The GMO Panel considers that for the comments related to lines 23-31, 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. For all the aspects raised in the comment, the GMO Panel refers the contributor to the related comments and responses provided, in particular for section 3.2.	15

Umweltbundesamt (Environment Agency Austria) on behalf of the Austrian lead Competent Authority, the Federal Ministry of Social Affairs, Health, Care and Consumer Protection.	Abstract	Line 17ff: The conclusion by the GMO Panel that, unlike for SDN-3 methods, the application of SDN-1, SDN-2, and ODM approaches results in the modification of plant endogenous genomic sequences without the insertion of exogenous DNA, is far too generalising. This is demonstrated by examples for introduction of foreign DNA during SDN-based genome editing, among others in cows (Norris et al. 2020) and oilseed rape (Braatz et al. 2017). During the genome editing process insertions of non-homologous DNA sequences may happen at the site or in their vicinity of SDN-induced double-strand breaks resulting in integration of short stretches of ectopic DNA (indels). Such ectopic insertions should not be disregarded as they may result in case-specific unintended effects comparable to the unintended effects due to the insertion of "foreign" recombinant DNA constructs during SDN-3 applications. Therefore the considerations of the opinion on SDN-3 are irrelevant only if a risk assessment has shown that plants obtained via SDN-1, SDN-2, and ODM approaches indeed do not contain foreign or ectopic DNA in the final product (Line 21-22). The abstract needs to be revised to take this into account. Line 23-24: Again, the overall conclusion that no new hazards exist that are specifically linked to the genomic modification produced via SDN-1, SDN-2 and ODM is too generalised. In this respect two groups of products need to be distinguished: • Genome editing applications which are comparable to products of natural variation or conventional breeding using induced mutagenesis (i.e. in short genetic changes at a single genomic locus, such as single base pair changes or random sequence changes) • Genome editing applications which are unlike products of convention-al breeding approaches (including products containing simultaneous genetic changes at multiple loci or longer non-random sequence changes (two or more base-pairs) which are not already occurring in existing plant plants that are sexually compatible with the plant species to be modified). These genome editing products are not only unlike conventional products in their pattern of genetic modification, such products also cannot be readily generated by the current practice of conventional plant breeding (Duensing et al., 2018). Unlike the draft opinion at hand a recent rule of USDA-APHIS in this respect is differentiating between both groups (USDA-APHIS, 2020). SDN-1, SDN-2 and ODM applications containing two or more separate genetic modifications are not exempt from regulation and have to undergo the standard regulatory procedure, including case-specific risk assessment as applicable. Line 28-31: Such a conclusion needs to be based on a robust demonstration that the final products obtained via SDN-1, SDN-2, and ODM indeed do not contain foreign or ectopic DNA. This needs to be demonstrated by appropriate molecular characterisation during risk assessment, e.g. as described in Eckerstorfer et al. (2019a). Braatz, J., Harloff, H. J., Mascher, M., Stein, N., Himmelbach, A., and Jung, C. (2017). CRISPR-Cas9 targeted mutagenesis leads to simultaneous modification of different ho-moeologous gene copies in polyploid oilseed rape (Brassica napus). Plant Physiol. 174, 935–942. doi: 10.1104/pp.17.00426 Duensing, Nina; Sprink, Thorben; Parrott, Wayne A.; Fedorova, Maria; Lema, Martin A.; Wolt, Jeffrey D.; Bartsch, Detlef (2018): Novel Features and Con-siderations for ERA and Regulation of Crops Produced by Genome Editing. Frontiers in bioengineering and bio-technology 6, S. 79. DOI: 10.3389/fbioe.2018.00079. Eckerstorfer, Michael F.; Dolezel, Marion; Heissenberger, Andreas; Miklau, Marianne; Reichenbecher, Wolfram; Steinbrecher, Ricarda A.; Waßmann, Friedrich (2019a): An EU Perspective on Biosafety	The GMO Panel thanks for the comment. Please note that the abstract summarizes the opinion by specifically addressing the terms of reference provided by the European Commission. Regarding the unintended integration of exogenous DNA (line 17ff and 28-31), the opinion already includes specific considerations in section 3.2.2.2.2. Regarding comment to lines 23-24, please note that a series of considerations on the type of modification introduced in the plant genome is included in section 3.2.2.1 of the opinion.	16

		Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs). Frontiers in bioengineering and bio-technology 7, S. 31. DOI: 10.3389/fbioe.2019.00031. Norris AL, Lee SS, Greenlees KJ, Tadesse DA, Miller MF, Lombardi HA (2020) Template plasmid integration in germline genome-edited cattle. Nat Biotechnol 38 (2):163-164. doi:10.1038/s41587-019-0394-6 USDA-APHIS (2020): Amendment of 7 CFR Parts 330, 340, and 372, Docket No. APHIS-2018-0034, RIN 0579-AE47		
International Seed Federation	Abstract	The International Seed Federation (ISF) is a non-governmental, non-profit organization. ISF represents more than 7500 seed companies active in breeding, seed production and trading and is widely regarded as the voice of the global seed industry. One of the primary objectives of ISF is to facilitate the movement of seed within a framework of fair and science-based regulations, whilst serving the interests of farmers, growers, industry and consumers. ISF believes that the adoption of science-based, consistent policies for products of the latest plant breeding methods, such as genome editing, will facilitate the development and uptake of advanced, innovative breeding applications by private and public breeders in developed and developing countries. ISF welcomes the efforts of the EFSA to clarify its approach to safety aspects of genome editing and the opportunity to provide comments. ISF is pleased with this very comprehensive and well balanced scientific analysis. ISF agrees with the overall conclusion of the panel that didn't identify new hazards specifically linked to the application of SDN-1, SDN-2 and ODM as compared to both SDN-3 and conventional breeding.	The GMO Panel thanks ISF for the comment.	17
Cornell University's Alliance for Science	Abstract	The Cornell Alliance for Science (AFS) appreciates the opportunity to submit comments on EFSA's GMO Panel's (The Panel) draft scientific opinion on the applicability of the European Food Safety Authority (EFSA) opinion on site-directed nucleases type 3 (SDN-3) for the safety assessment of plants developed using site-directed nucleases type 1 and 2 (SDN-1 and SDN-2) and oligonucleotide-directed mutagenesis (ODM). We support science and risk-based oversight by EFSA of gene-edited plants to ensure they are safe to humans and the environment. We believe that The Panel is correct in limiting how much of the "EFSA's opinion on SDN-3" applies to the risk assessment of plants generated by SDN-1, SDN-2 and ODM techniques. However, additional clarity should be provided on how to implement the portions of the opinion that The Panel considers are relevant. The AFS agrees with The Panel's assessment in finding no additional hazard associated to the use of the SDN-1, SDN-2 and ODM approaches as compared to both SDN-3 and conventional breeding techniques, including conventional mutagenesis. The Panel's analysis also accurately supports significantly reduced oversight for any plants obtained through these techniques that do not contain exogenous DNA. AFS promotes applying different risk assessment requirements to SDN1, SDN2, and	The GMO Panel thanks for the comment.	18

		ODM produced plants based on the risk-proportion of the final product obtained on a case-by-case basis.		
GenØk-centre for biosafety	Abstract	The EFSA GMO panel was asked by EFSA to assess whether section 4 (hazard id) and conclusions valid for SDN-3 are valid for SDN-1, SDN-2 and ODM plants. They concluded that there is no inclusion of exogenous DNA by application of these three methods and that Guidance for risk assessment of food and feed from GM plants are sufficient and can be partly applied. The GMO panel did however not consider the off-target effects caused by these methods, as has been demonstrated in several studies already (after cutting in genomic DNA, repair mechanism introduces changes in sequence, dependent on method in use. These are mutations like insertions, substitutions, deletions etc . See the review Han et al 2020 " Mitigating off-target effects ..." Journal of medicine 98:615-32 for more information). The introduction of exogenous DNA by use of TALEN in the case of hornless cattle where template DNA was found integrated in the genome (Norris et al 2020, "Template plasmid integration in germline genome-edited cattle", Nature Biotechnology, 38:163-4) shows that guidance requirements for investigating and assessing presence of exogenous DNA, must be included for gene-edited plants also.	The GMO Panel thanks for the comment. Please note that the abstract summarizes the opinion by specifically addressing the terms of reference provided by the European Commission. Regarding the unintended integration of exogenous DNA, the opinion already includes specific considerations in section 3.2.2.2.2. Please also note that a series of considerations on the type of modification introduced in the plant genome is included in section 3.2.2.1 of the opinion.	19
GMWatch	Abstract	Lines 10-12 EFSA's 2012 Opinion on the risk assessment of plants developed with SDN-3 needs to be rewritten to take into account relevant studies; it is not a sound basis for argument in this current draft document. e.g. EFSA's statement, "The main difference between the SDN-3 technique and transgenesis is that the insertion of DNA is targeted to a predefined region of the genome. Therefore, the SDN-3 technique can minimise hazards associated with the disruption of genes and/or regulatory elements in the recipient genome", is outdated. It – and the current EFSA draft document – does not take account of studies such as those in the "List of studies" included in this submission, which show unexpected on-target and off-target effects from gene editing. The 2012 Opinion also says (this conclusion is repeated in the draft document), "Whilst the SDN-3 technique can induce off-target changes in the genome of the recipient plant these would be fewer than those occurring with most mutagenesis techniques. Furthermore, where such changes occur they would be of the same types as those produced by conventional breeding techniques." This conclusion is unreliable, since: 1) Many recent studies show that unintended on-target effects and off-target effects of gene editing have been missed due to inadequate screening (e.g. Shin HY et al (2017) https://www.ncbi.nlm.nih.gov/pubmed/28561021; Hahn F, Nekrasov V (2018) https://link.springer.com/article/10.1007/s00299-018-2355-9). 2) It is not just the number of off-target changes that are of concern; it is where they are located and what they do (quality). Even if off-target mutations arising from gene editing are fewer in number compared to other methods, they are not random but at genome locations similar in sequence to the intended target site. This carries with it a greater risk of disturbing gene function than can result from random mutagenesis. It is the responsibility of EFSA to assess the effects of these changes. If insufficient data exists to evaluate this, EFSA must say so.	Regarding comment to lines 10-12, and 17-25, the GMO Panel refers the contributor to the related comments and responses provided, in particular related to section 3.2 of the opinion.	20

		3) It is not valid to compare SDN-3 with random mutagenesis as practised in conventional breeding. The GMO legislation states that random mutagenesis has a history of safe use; such a history is lacking in the case of gene-editing applied to food plants. 4) It is not valid to conclude that SDN-3 techniques would produce changes "of the same types as those produced by conventional breeding techniques" (e.g. random mutagenesis). The body of scientific data that would enable such a conclusion – for example, molecular analysis of a variety of randomly mutagenized crops compared with SDN-3-produced crops – does not exist. EFSA fails to consider that the gene-editing techniques can bypass limits of gene recombination and regulation that exist in conventional breeding and can cause patterns of genetic change and new combinations of genetic information which cannot be achieved via conventional breeding, including random mutagenesis (Kawall K (2019), https://www.frontiersin.org/articles/10.3389/fpls.2019.00525/full). Lines 17-25 The "List of studies" in the attached file shows that the risks of gene editing are not restricted to insertion of "exogenous DNA" (SDN-3), but also relate to gene disruption (SDN-1) and modification (SDN-2). All types of SDN are genetic modification procedures that have unintended outcomes, at both intended on-target and unintended off-target locations within the genome. In the case of crops produced with SDN procedures, on-target deletions or rearrangements and/or off-target effects could disrupt the function of multiple genes, which could lead to altered biochemistry and hence unexpected toxicity or allergenicity, or undesirable environmental impacts. Unintended on-target or off-target effects can result from modifications described as small; that is, where one or a few nucleotides of a gene have been altered. Even changing a single nucleotide within a gene's sequence can induce drastic changes in the function of its RNA or protein product and/or its expression. Such changes can be brought about in SDN1, SDN2, and SDN3 procedures. For example, a change in the function of an enzyme through alteration of its active site can lead to its being able to carry out unintended biochemical reactions. In addition, genes and their RNA or protein products work in networks. An apparently small change in one gene can affect the function of other members of the network. It should also be considered that while DNA-free gene editing is possible, to date most gene-edited plants have been developed via insertion of a cassette containing DNA coding for the gene-editing components. This insertion can cause fragments and rearrangements, in addition to unintended effects caused by the gene-editing process. This totality of unintended effects must be considered in the risk assessment.		
Envirnonmental association Za Zemiata	Abstract	[line 23 after first bullet, up to line 31: Delete and replace text:] "However, even if no additional DNA is inserted, the specific pattern of intended and unintended genetic changes caused by SDN-1 and SDN-2 applications needs to be assessed case by case. SDN applications have to be regarded as biological mutagens that, unlike chemical or physical mutagens, can directly and specifically interact with the biological mechanisms in the cell on the level of the genome and/or epigenome. These applications have a high potential to penetrate the genome and generate profound alterations in the biological characteristics of plants without introducing any additional DNA sequences. These changes	The GMO Panel considers that for the comments related to lines 23-31, an explanation of the rationale for the proposed change is not sufficiently justified. Therefore, the proposed changes have not been	21

		will typically give rise to biological characteristics, such as changes in plant composition that exceed the range of characteristics achieved by previous plant breeding methods. The risks associated with the release, cultivation and consumption of these plants need to be fully investigated before any conclusions on the safety of the new organisms can be drawn. In addition, there is a wide range of specific on-target and off-target effects of SDN-1 and SDN-2 interventions, largely depending on various parameters of the specific technical processes such as: (i) the specific nuclease(s) used; (ii) the target organism and its tissue, respectively; (iii) the targeted gene(s); (iv) the way in which the components are introduced into the cells; (v) the dosage of the nuclease(s); (vi) if CRISPR/Cas is applied, the guide RNA used and (vii) duration of the intervention. All these technical details determine the precision as well as the efficiency of an intervention and also have to be taken into account during risk assessment. The methodology and guidance for risk assessment needs to be adopted accordingly, taking into account the restrictions associated with the comparative approach and complex challenges posed by SDN-1 and SDN-2 plants in environmental risk assessment. The requirements for a more detailed risk assessment are not solely linked to the presence of foreign DNA, but are relevant for all genome-edited plants, including those that were developed with SDN-1, SDN-2 and whether the genome of the final product does not contain any exogenous DNA. Since these issues are not, or only partially, addressed in the previous EFSA opinion on SDN-3 (EFSA 2012a), the methodology and conclusions derived in this earlier document are not sufficient to guide risk assessment of SDN-1 and SDN-2 applications. As far as ODM is concerned, the EFSA panel could not reach final conclusions, since there is a substantial lack of data on the side effects associated with the specific method. Whatever the case, detailed examination of an organism's genetic and overall biological characteristics, starting with the process that was used to introduce changes in the genome of the organism, is needed to decide whether the organism is safe. The set of data needed for risk assessment will be dependent on each case and cannot generally be limited by criteria such as the insertion of additional genes."	integrated in the text of the opinion. For all the aspects raised in the comment, the GMO Panel refers the contributor to the related comments and responses provided, in particular for section 3.2.	
Greenpeace European Unit	Abstract	The GMO Panel concludes that there are no "new hazards specifically linked to the genomic modification produced via SDN-1, SDN-2 and ODM as compared to both SDN-3 and conventional breeding". We agree that there are similarities in the safety considerations related to SDN-1, SDN-2 and ODM compared to those related to SDN-3, although there are also important differences. For example, SDN-1 – but not SDN-3 – can be used to obtain changes across different target sites in the genome (e.g. through multiplexing or serial applications), giving rise to additional safety concerns. This is an aspect the GMO Panel has so far failed to consider. We do not agree that safety considerations for genome editing as a whole (i.e. SDN-1, SDN-2, ODM and SDN-3) are similar to those related to "conventional breeding". A wide range of unintended changes in the genome have been documented in both SDN- and ODM-	The GMO Panel thanks for the comment. For all the aspects raised in the comment, the GMO Panel refers the contributor to the related comments and responses provided, in particular for section 3.2.	22

		based approaches. They cannot be compared to changes occurring in mutation breeding. The scientific evaluation of individual products should consider both intended and unintended genetic alterations arising from the complete processes of SDN and ODM applications (including unintended changes resulting from gene delivery and integration, DNA, RNA or protein delivery processes and cell culture), as well as their potential safety implications.		
BUND e.V. / Friends of the Earth Germany	Abstract	Line 23: ADD between "product" and "overall": ";though there are findings in literature showing that different methods of inserting the CRISPR/Cas-complex can result in fragments of foreign DNA remaining in the final product (Andersson et al. 2018, Jupe et al. 2019)". Line 29: ADD after "DNA": ", but there is still the need to thoroughly assess specific risks linked to the use of SDN-1 and SDN-2 including the (unintended) presence of foreign DNA. As for ODM, there is too little data to compare the special risks of its use. Though fully recognizing the advantages of the European food and feed risk assesment on GMO, there is evidence in the literature of risks, especially to the environment, not sufficiently covered so far (e.g. Hilbeck et al. 2015, 2020). Those must be addressed as well in assessing SDN-1 and SDN-2." Additional Comment: As pointed out in the following additions and changes, we do see the need of a specific risk assessment of SDN-1 and SDN-2 applications, since the risks of their use such as on- and off-target-effects seem not to be taken fully into account by EFSA. Since this EFSA-opinion only compared SDN-1 and SDN-2 to risks identified with SDN-3 and recommendations derived from that, it cannot provide an answer to specific risks of SDN-1 and SDN-2 applications, neither of ODM. This must be clearly indicated in the opinion. Besides, there are new publications as (e.g. Agapito-Tenfen et al. 2018; Cotter et al. 2020; Eckerstorfer et al. 2019; Hahn & Nekrasov 2019; Kawall 2019; Wolt et al. 2016; Zhu et al. 2017)which indicate the need of a revision of the 2012 – opinion on SDN-3. An opinion on SDN-1 and SDN-2 still must take newer findings into account, esp. concerning those risks not solely linked to the presence of foreign DNA, which are not sufficiently addressed in EFSA-opinion on SDN-3. Additionally, the opinion should cover recommendations to risk assessments regarding risks to the environment which have been shown in newer publications (e.g. Hilbeck et al. 2015, 2020) - or the opinion should clearly state which risks (e.g. to the environment) have not been assessed by 2012, and therefore are not part of this opinion.	The GMO Panel considers the text of the abstract in line with the content of the main text of the opinion which has been revised based on the comments received. Regarding the unintended integration of exogenous DNA, the opinion already includes specific considerations in section 3.2.2.2.2. Please note that a series of considerations on the type of modification introduced in the plant genome is included in section 3.2.2.1 of the opinion.	23
Testbiotech	Keywords	[Add]: biological mutagen, pattern of genetic changes, comparative approach, transcriptomics, proteomics, metabolomics, whole genome sequencing, environmental risk assessment, risk scenarios, unintended on-target effects.	The GMO Panel considers the keywords' list exhaustive.	24
Federal Agency for Nature Conservation	Keywords	Line 36-37: Add on-target or on-target damage as a keyword.	The GMO Panel considers the keywords' list exhaustive.	25
Federal Agency for Nature Conservation	Keywords	Line 36-37: Add on-target or on-target damage as a keyword.	The GMO Panel considers the keywords' list exhaustive.	26
ENSSER	Introduction	Missing: • Referring to EU's stance/position on the PP – as a foundation and guidance for this opinion and the GM issue	The text has been provided by the European Commission in the frame of the mandate's official documentation.	27

ENSSER	1.1 Background as provided by the European Commission	Line 75: mutagenesis techniques that have "emerged" since the adoption of the Directive. Apologies, where in the ruling does it say that? Does it not rather refer to those that are excempt because they "conventionally been used in a number of applications and have a long safety record"(ECJ). (or commonly referred to as having a history of safe use)? Line 85: Which part of mutagenesis is it compared to?? "Among the conventional plant breeding techniques, the EFSA GMO Panel considered certain mutation breeding techniques that emerged before the adoption of the Directive 2001/18/EC and that are used as a tool to create genetic variation." Line 86: Which techniques are being referred to when talking about conventional breeding techniques and which are the mutation breeding techniques that are being considered? Please also specify if "emerged before" the adoptation of the Directive 2001/18/EC also means 'having a long history of safe use ……. …" Line 88: Beginning of quoted para of the opinion is missing. The 2012 opinion itself already has points of concern, that we think need to be adressed in this opinion, and should not just be copied at verbatum and regarded as true/scientifically accurate. Given 8 years of difference, more knowledge has come to light, but more still needs to be obtained. • Please explain how is it known that SDN-3 "minimize" hazards and provide the data and scientific evidence. Whilst this may well be the intention, it is the actual practice and robust data that count. • Off-targets would be "fewer" "than those occurring with most mutagenesis techniques". Again, there is no literature and data to evidence this is the case, and that considering and include all processes involved and investigate and account for all unintended mutations. • "they would be of the same types as those produced by conventional breeding techniques" Please explain what is meant by 'same types'? Is that intended as an assurance of safety? Is "same" meaning to be indels, deletions, alterations, small, sometimes large. Or is it in the same regions? Please provide the science to evidence this. Line 95: "however, on a case by case basis, lesser … may be needed" Please provide details and guidance as to when this would be the case, and what the "lesser" refers to. If there is no guidance available it will need to be flagged up in the conclusions as task to undertake.	Regarding comment for line 75, the text has been provided by the Euopean Commission in the frame of the mandate's official documentation. The C-528/16-Judgment (summary) point 2 states that: "[…]article 3(1) of Directive 2001/18, read in conjunction with point 1 of Annex I B to that directive, cannot be interpreted as excluding, from the scope of the directive, organisms obtained by means of new techniques/methods of mutagenesis which have appeared or have been mostly developed since Directive 2001/18 was adopted[…]". The specific text included in this opinion can be found in the press release No 111/18 (Luxembourg, 25 July 2018). Regarding comment for line 85 and 86, the text has been provided by the Euopean Commission in the frame of the mandate's official documentation. The techniques are mutagenesis approaches which include spontaneous and induced mutations (the latter include chemical and physical mutagenesis and somaclonal variation). Regarding comment for line 88, the GMO Panel would like to clarify that this specific text has been provided by the European Commission in the frame of the mandate's official documentation. Regarding all the comments raised in the bullet points (off-targets), the GMO Panel invites ENSSER to refer to the specific sections in the opinion (section 3.2.2 and related	28

			subsections) and to the responses given to the comments related to those sections. Regarding comment for line 95, the GMO Panel invites ENSSER to refer to the specific sections in the opinion (section 3.3 and 4) and to the responses given to the comments related to those sections.	
Logos Environmental	1.1 Background as provided by the European Commission	Ln 88-92. The conclusion of the 2012 opinion on SDN3 needs to be revised in light of new publications (see, e.g. Agapito-Tenfen et al. (2018) Front. Plant. Sci. 9: 1874; Cotter et al. (2020) www.testbiotech.org/en/content/rages-subreport-new-genetic-engineering-technologies; Eckerstorfer et al. (2019). Front. Bioeng. Biotechnol. 7: 31; Hahn & Nekrasov (2019) Plant Cell Rep. 38: 437 441; Kawall (2019) Front. Plant Sci. 10, 525; Wolt et al. (2016) Plant Genome 9: 1 8; Zhu et al. (2017) Trends Plant Sci. 22: 38–5). There was little evidence to support this in the opinion in 2012, and this conclusion is even less solid now, to the point of being incorrect.	Regarding comment for line 88-92, the GMO Panel would like to clarify that this specific text has been provided by the European Commission in the frame of the mandate's official documentation. On the off-target mutations related to SDN-based approaches, the GMO Panel invites Logos Environmental to refer to the specific sections in the opinion (section 3.2.2 and related subsections) and to the responses given to the comments related to those sections.	29
Association Française de Biotechnologies Végétales	1.1 Background as provided by the European Commission	AFBV comments: We propose (1) edits to the text to improve its clarity and (2) comments directed to the contents of the scientific opinion. Line numbers are those of the .pdf version downloaded from the EFSA site. In the text above we suggest the following edits: • Line 82: replace "In this scientific opinion" with "In the SDN-3 Scientific Opinion"; • Line 88: replace "The scientific opinion" with "The SDN-3 Scientific Opinion"; • Line 93: after "also concluded" insert ", in its SDN-3 Scientific Opinion,"; • Footnote #3: insert at the end: "(hereinafter "SDN-3 Scientific Opinion)".	Regarding comment for line 82,88,and 93, the GMO Panel would like to clarify that this specific text has been provided by the European Commission in the frame of the mandate's official documentation. Regarding the comment for footnote #3, "hereinafter "EFSA opinion on SDN-3" has been added to keep consistency with the rest of the draft opinion.	30
Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG)	1.1 Background as provided by the European Commission	WGG agrees with the statement in lines 88 - 92	The GMO Panel thanks for the comment.	31

European Coordination Via Campesina	1.1 Background as provided by the European Commission	Ln 88-92. The conclusion of the 2012 opinion on SDN3 needs to be revised in light of new publications. In general, the safety of new genomic techniques has not been evaluated and scientific studies show that these techniques result in unexpected concerning alterations of the genome, both at the intended target and off-target sites. Any of these alterations could result in unexpected toxicity and/or allergenicity. The lack of knowledge also relates to the environmental and cumulative effects that may result from the products of these techniques. The following is an overview of scientific studies on these issues: https://www.gmwatch.org/en/news/latest-news/19223	Regarding comment for line 88-92, the GMO Panel would like to clarify that this specific text has been provided by the European Commission in the frame of the mandate's official documentation. On the off-target mutations related to SDN-based approaches, the GMO Panel invites the contributor to refer to the specific sections in the opinion (section 3.2.2 and related subsections) and to the responses given to the comments related to those sections.	32
National Food Institute, Technical University of Denmark	1.1 Background as provided by the European Commission	The scope of this opinion on SDN-1/SDN-2 greatly overlaps with the assessment given in the opinion on Synbio plants engineered with genome editing. It is unclear whether the outcome of these two opinions have been coordinated. E.g. in the SynBio plant opinion it is mentioned how protein levels and the genetic stability of the nucleotide change would have to be demonstrated, which is not mentioned in this opinion. The fact that there is now one separate opinion where SDN-1/SDN-2 is compared with SDN-3 and another separate opinion where SDN-1/SDN-2 is compared with traditional GMO is confusing. It would be advisable to create a single opinion on SDN-1 and SDN-2, where an assessment was made comparing both to the GMO guidelines as well as to the procedure for plants developed with conventional mutagenesis.	The GMO Panel thanks for the comment. The GMO panel was mandated by the EC to develop several indepentt opinions. Where appropriate, the development of opinions was coordinated between working groups. All the scientific opinions are aligned and have been approved by the EFSA GMO Panel. Specifically, SynBio Plants opinion is much broader than SDN-1, SDN-2 and ODM opinion. However, one of the case studies indeed represents a relatively complicated SDN-2 scenario. While for SDN-1, SDN-2 and ODM applications, in general, less information may be required, for specific cases, such as genome-edited wheat more specific data requirements may be adopted.	33
COST Action CA18111 - Plant genome editing – a technology with transformative potential (PlantEd)	1.1 Background as provided by the European Commission	L. 77: The objective of Directive 2001/18/EC "is to approximate the laws, regulations and administrative provisions of the Member States and to protect human health and the environment when carrying out the deliberate release into the environment of genetically modified organisms for any other purposes than placing on the market within the Community, [and] placing on the market genetically modified organisms as or in products within the Community." We suggest the sentence concerned to be rephrased in order to capture the complete intention of Directive 2001/18/EC.	Regarding comment for line 77 and 84/85, the GMO Panel would like to clarify that this specific text has been provided by the European Commission in the frame of the mandate's official documentation. The GMO Panel	34

		L. 84/85: PlantEd suggests rephrasing line 86 as follows to further clarify what is referred to by conventional mutation breeding techniques: "…the EFSA GMO Panel considered in vivo and in vitro mutation breeding techniques that emerged before the…" The clarification should implement the European Court of Justice's interpretation of the term "mutagenesis" in the Directive to exclude those methods/technologies developed primarily after 2001 " (for a discussion of the Court ruling, see Purnhagen et al, 2018; Vives-Vallés and Collonnier, 2020).	developed the opinion by strictly adhering to the terms of references provided by the European Commission. Providing further interpretation of the judgement of the Court of Justice of the European Union (CJEU) in Case C-528/16 on mutagenesis and/or of the Directive 2001/18 is not in the remit of the GMO Panel.	
French agency for Food, Environmental and Occupational Health & Safety (Anses)	1.1 Background as provided by the European Commission	Page 3, lines 73-76: The precise formulation in the judgement of the Court of Justice of the European Union in Case C-528/16 is: "In the light of the foregoing considerations, the answer to the first question is as follows: [...] Article 3(1) of Directive 2001/18 [...] must be interpreted as meaning that only organisms obtained by means of techniques/methods of mutagenesis which have conventionally been used in a number of applications and have a long safety record are excluded from the scope of that directive." The exact list of techniques that meet the criteria "which have conventionally been used in a number of applications and have a long safety record" is not yet established. Additionally, the precise expression in the recitals 47 and 51 is "which have appeared or have been mostly developed since Directive 2001/18 was adopted". Therefore, this sentence should be rephrased, to be more exact (e.g. "The judgement of the Court of Justice [...] has clarified that only organisms obtained by means of techniques/methods of mutagenesis which have conventionally been used in a number of applications and have a long safety record are excluded from the scope of Directive 2001/18/EC. The exact list of techniques that meet this criteria is not yet established, but it can be anticipated that SDN-1, SDN-2, and ODM techniques ("new mutagenesis techniques"), which have appeared or have been mostly developed since Directive 2001/18 was adopted, will come within the scope of that directive."). Page 3, lines 83-84: "to compare the hazards associated with plants produced by the SDN-3 technique with those obtained by conventional plant breeding": this wording doesn't seem correct because the hazards are not obtained by conventional plant breeding. Proposal to rephrase it as follows: "to compare the hazards associated with plants produced by the SDN-3 technique with those of plants obtained by conventional breeding". Page 3, lines 89-92: "Whilst the SDN-3 technique can induce off-target changes in the genome of the recipient plant, these would be fewer than those occurring with most mutagenesis techniques. Furthermore, where such changes occur, they would be of the same types as those produced by conventional breeding techniques.": the fact that the off-target changes would be fewer must be referenced or analysed. Additionally, even if they are fewer and of the same type as those produced by conventional breeding techniques, this is not enough to exclude the occurrence of new hazards, which need to be studied. Page 3, lines 93-95: "The EFSA GMO Panel also concluded that its 2010 and 2011 guidance documents "are applicable for the evaluation of food and feed products derived from plants developed using the SDN-3 technique and for performing an environmental risk assessment. [...]"": this is not true for the analysis of potential off-target effects (same comment as on lines 25-27).	Regarding comment for line 73-76, 83-84, 89-92, and 93-95, the GMO Panel would like to clarify that this specific text has been provided by the European Commission in the frame of the mandate's official documentation. Regarding the off-target mutations (comment for lines 89-92) and the applicability of the EFSA guidances (comment for line 93-95), the GMO Panel invites ANSES to refer to the specific sections in the opinion (section 3.2.2 and related subsections, section 3.3, and conclusions) and to the responses given to the comments related to those sections.	35
Nature et Progrès Belgique	1.1 Background as provided	Ln 88-92. The conclusion of the 2002 opinion on SDN3 needs to be revised in light on new publications (see, e;g Agapito-Tenfen et al (2018) Front.Plant.Sci.9:1874; Cotter et al (2020)	Regarding comment for line 88-92, the GMO Panel would like to clarify	36

	by the European Commission	www.testbiotech.org/en/content/rages-subreport-new-genetic-engineering-technologies; Eckerstorfer et al (2019). Front.Bioeng.Biotechnol.7:31; Hahn & Nekrasov (2019) Plant Cell Rep. 38:437-441; Kawall (2019) Front Plant Sci.10, 525; Wolt et al (2016) Plant Genome 9:18; Zhu et al (2017) Trends Plant Sci.22:38-5). There was little evidence to support this in the opinion in 2012, and this conclusion is even less solid now, to the point of being incorrect.	that this specific text has been provided by the European Commission in the frame of the mandate's official documentation. On the off-target mutations related to SDN-based approaches, the GMO Panel invites the contributor to refer to the specific sections in the opinion (section 3.2.2 and related subsections) and to the responses given to the comments related to those sections.	
Nature et Progrès Belgique	1.1 Background as provided by the European Commission	Ln 88-92. The conclusion of the 2012 opinion on SDN3 needs to be revised in light of new pubications (see, e.g>. Agapito-Tenfen et al (2018) Front.Plant.Sci. 9:1874; Cotter et al (2020) www.testbiotech.org/en/content/rages-subreport-new-genetoic-engineering-technologies; Eckerstorfer et al (2019). Front.Bioeng.Biotechnol. 7:31; Hahn & Nekrasov (2019) Plant Cell Rep. 38: 437-441; Kawall (2019) Front.Plant.Sci. 10, 525; Wolt et al (2016) Plant genome 9: 1 8; Zhu et al (2017) Trends Plant Sci.22: 38-5). There was little evidence to support this in the opinion in 2012, and this conclusion is even less solid now, to the point of being incorrect.	Regarding comment for line 88-92, the GMO Panel would like to clarify that this specific text has been provided by the European Commission in the frame of the mandate's official documentation. On the off-target mutations related to SDN-based approaches, the GMO Panel invites the contributor to refer to the specific sections in the opinion (section 3.2.2 and related subsections) and to the responses given to the comments related to those sections.	37
Haut Conseil des biotechnologies (High Council for Biotechnology)	1.1 Background as provided by the European Commission	It is not clear whether the text of this "background as provided by the European Commission" can be modified. If it can be modified, we suggest the following corrections. If it is a citation that cannot be modified, we suggest having it written in quotation marks or in italics, and adding clarifying comments in footnotes. l. 73-76. The terms "have emerged since its adoption" do not accurately reflect the conclusions of the CJEU judgement of 25 July 2018 in Case C-528/16 and may be misleading as a result. To avoid any risk of misinterpretation, we recommend keeping as close as possible to the exact wording of the CJEU judgement, as follows: "The judgement of the Court of Justice of the European Union (CJEU) in Case C-528/16 on mutagenesis has clarified that "organisms obtained by means of techniques/methods of mutagenesis constitute genetically modified organisms within the meaning of [Directive 2001/18/EC]" and that "only organisms obtained by means of techniques/methods of mutagenesis which have conventionally been used in a number of applications and have a long safety record are excluded from the scope of that directive" (Article 1). It follows that Directive 2001/18/EC is applicable to GMOs obtained by	The GMO Panel would like to clarify that the text in section 1.1 has been provided by the European Commission in the frame of the mandate's official documentation. The GMO Panel thanks for the suggestion; a clarifying comment in the footnote has been inserted. Regarding the conventional breeding techniques, the text of the opinion has been revised to improve its clarity. In particular, section 2.1.1 and footnote 5 have been amended accordingly.	38

		SDN-1, SDN-2 and ODM techniques". l. 84-85. The terms "conventional breeding techniques" can be confusing. The definition is only given in footnote 5 page 5. For the sake of clarity, we suggest defining it here, when it is first used in the opinion, at least in the form of a footnote. Furthermore, we suggest modifying the definition from footnote 5 as follows: Instead of the current text ("Conventional plant breeding is defined as methods used by plant breeders for the improvement of commercial varieties and where the resulting plants/varieties are not covered by the legal definitions of genetic modification (Directive 2001/18/EC)"), which may be interpreted as excluding the techniques listed in Annex IB from what is considered as conventional breeding techniques, we suggest: "Conventional plant breeding is defined as methods used by plant breeders for the improvement of commercial varieties and where the resulting plants/varieties do not fall within the scope of Directive 2001/18/EC, either because they do not fall under the legal definition for a GMO, or because they do but they are exempted from application of the Directive". l. 85-87. "Among the conventional plant breeding techniques, the EFSA GMO Panel considered certain mutation breeding techniques that emerged before the adoption of the Directive 2001/18/EC and that are used as a tool to create genetic variation. » Could the techniques that were considered by the GMO Panel be specified for clarity?		
GenØk-centre for biosafety	1.1 Background as provided by the European Commission	The conclusion that SDN-3 technique can minimize hazards associated with the disruption of genes and/or regulatory elements in the recipient genome lacks references for the statement " where such change occur, the mutations will be the same as those produced by conventional breeding techniques". Do the mutations happen at the same place(s), are they random or are they connected to certain repair mechanisms? For details regarding this section: please read our attached table with our comments. Copied from submitted pdf file: of the genome that they involve would result in the same effects as the introduction of a foreign gene, specific to transgenesis. In addition, since the development of the new techniques of mutagenesis allows the production of modifications of the genetic heritage to increase at a rate out of all proportion to the modifications likely to occur naturally or randomly, the possibility of harm occurring as a result of unintentional modifications of the genome or of the properties of the plant thus obtained would be increased."1 These issues raised by ECJ are directly linked to EFSA mandate and ToR but were not included neither discussed within the document. Conventional mutagenesis is not conventional breeding It is also relevant to clarify in this section that random or chemical mutagenesis are genetic engineering techniques and not conventional breeding techniques. The reason why these techniques are not regulated by the GMO Directive is because of their history of safe use which exempts them	The GMO Panel would like to clarify that the text in section 1.1 has been provided by the European Commission in the frame of the mandate's official documentation and it refers to the conclusions of the EFSA opinion on SDN-3. On the off-target mutations related to SDN-based approaches, the GMO Panel invites the contributor to refer to the specific sections in the opinion (section 3.2.2 and related subsections) and to the responses given to the comments related to those sections.	39

		from being regulated. There is a clear distinction between what the Court considers conventional breeding techniques and genetic engineering techniques exempt from regulation. EFSA provides a correct definition of conventional breeding in footnote #5 – "Conventional plant breeding is defined as methods used by plant breeders for the improvement of commercial varieties and where the resulting plants/varieties are not covered by the legal definitions of genetic modification". However, it is incorrect when it includes conventional mutagenesis techniques as conventional breeding techniques. Mutagenesis techniques are covered by the Directive. EFSA interpretation of mutagenesis techniques is flawed and misleading as it is not clear when it refers to risks related to conventional breeding (crossing and selection of genotypes) and when it refers to risks of chemical mutagenesis. Such flawed statements should be corrected throughout the document: Page 1 - Lines 17 and 24 Page 4 - Lines 84, 85 and 92 Page 5 – Line 135 Page 6 - Lines 144, 154, 167 and 175 Page 10 – Line 279 Page 11 - Lines 341, 347 and 353 Page 12 – Lines 374 and 387 Page 13 – Lines 417 and 418		
Federal Agency for Nature Conservation	1.1 Background as provided by the European Commission	Lines 85-92: The draft describes correctly that mutation breeding techniques which emerged before the adoption of Directive 2001/18/EC (as well as of course conventional breeding) are used (as a tool) to create genetic variation. In conventional breeding genetic variation is largely and intentionally increased in a first step followed by selection of individual plants with a desired phenotypic trait (often aided by molecular diagnosis tools) and backcrossing in the subsequent steps. Unlike with SDN techniques often there is no distinct molecular target in conventional breeding and therefore and in this respect there are no off-target effects. The draft and the EFSA opinion on SDN-3 consider that mutations deriving from conventional breeding, mutagenesis or SDNs are principally similar. This disregards that the genome becomes more accessible for changes by genome editing compared to conventional breeding (Kawall 2019) and that genome editing can alter several copies of a gene within a genome (e.g. Kannan et al. 2018) (see also comment to 2.1.3). Kannan, Baskaran; Jung, Je Hyeong; Moxley, Geoffrey W.; Lee, Sun-Mi; Altpeter, Fredy (2018): TALEN-mediated targeted mutagenesis of more than 100 COMT copies/alleles in highly polyploid sugarcane improves saccharification efficiency without compromising biomass yield. In: Plant Biotechnol J 16 (4), p. 856–866. DOI: 10.1111/pbi.12833. Kawall, Katharina (2019): New Possibilities on the Horizon: Genome Editing Makes the Whole Genome Accessible for Changes. In: Front. Plant Sci. 10, p. 280. DOI: 10.3389/fpls.2019.00525.	Regarding the comment for line 85-92 and more specifically on the off-target mutations related to SDN-based approaches, the GMO Panel invites the contributor to refer to the specific sections in the opinion (section 3.2.2 and related subsections) and to the responses given to the comments related to those sections.	40
Envirnonmental association Za Zemiata	1.1 Background as provided by the European Commission	Ln 88-92. The conclusion of the 2012 opinion on SDN3 needs to be revised in light of new publications (see, e.g. Agapito-Tenfen et al. (2018) Front. Plant. Sci. 9: 1874; Cotter et al. (2020) www.testbiotech.org/en/content/rages-subreport-new-genetic-engineering-technologies; Eckerstorfer et al. (2019). Front. Bioeng. Biotechnol. 7: 31; Hahn & Nekrasov (2019) Plant Cell Rep. 38: 437 441; Kawall (2019) Front. Plant Sci. 10, 525; Wolt et al. (2016) Plant Genome 9: 1 8; Zhu et al. (2017)	Regarding comment for line 88-92, the GMO Panel would like to clarify that this specific text has been provided by the European Commission in the frame of the mandate's official documentation.	41

		Trends Plant Sci. 22: 38–5). There was little evidence to support this in the opinion in 2012, and this conclusion is even less solid now, to the point of being incorrect.	On the off-target mutations related to SDN-based approaches, the GMO Panel invites the contributor to refer to the specific sections in the opinion (section 3.2.2 and related subsections) and to the responses given to the comments related to those sections.	
Corporate Europe Observatory	1.1 Background as provided by the European Commission	Ln 82-85 It is confusing to use the term "conventional breeding" as equalling mutagenesis by chemicals or radiation. Conventional breeding is a much wider term than that. Using "currently used transgenesis" is confusing as a term, since current GMOs have been generated by techniques that not necessarily produce transgenic organisms. This is because when using those techniques, it is equally possible to use genetic material from the same related species. Ln 88-92. It seems that since the 2012 opinion on SDN3 new publications have been published which need to be taken into account. For instance: Agapito-Tenfen et al. (2018) Front. Plant. Sci. 9: 1874; Cotter et al. (2020) www.testbiotech.org/en/content/rages-subreport-new-genetic-engineering-technologies; Eckerstorfer et al. (2019). Front. Bioeng. Biotechnol. 7: 31; Hahn & Nekrasov (2019) Plant Cell Rep. 38: 437 441; Kawall (2019) Front. Plant Sci. 10, 525; Wolt et al. (2016) Plant Genome 9: 1 8; Zhu et al. (2017) Trends Plant Sci. 22: 38–5). Many of these publications cannot be found in the current draft opinion at all. Please provide a reason if any of these publications is not considered.	The GMO Panel would like to clarify that the text in section 1.1 has been provided by the European Commission in the frame of the mandate's official documentation. Regarding the conventional breeding techniques, the text of the opinion has been revised to improve its clarity. In particular, section 2.1.1 and footnote 5 have been amended accordingly. Regarding the publications listed in comment for line 88-92, the GMO Panel was not mandated to produce a comprehensive literature review on SDNbase genome editing. In developing the scientific opinion, the GMO Panel took into consideration not only opinion papers but also research papers that provided actual experimental data on several aspects included in the opinion. Relevant publications deemed necessary have been included in the opinion, one of which is actually listed in the comment to line 88-92.	42
BUND e.V. / Friends of the Earth Germany	1.1 Background as provided by the European Commission	Line 91: ADD after "techniques" In the light of new data this conclusion is no longer valid (e.g. Kawall 2019, Agapito-Tenfen et al. 2018).	The GMO Panel would like to clarify that the text in section 1.1 has been provided by the European Commission in the frame of the mandate's official documentation. Regarding the conclusion in line 91 which refers	43

			to the potential off-target mutations, the GMO Panel invites the contributor to refer to the specific sections in the opinion (section 3.2.2 and related subsections) and to the responses given to the comments related to those sections.	
ENSSER	1.2 Background as provided by EFSA	We do not understand why this the background "as provided by EFSA"? Is it not the section above as well that is provided by EFSA, the opinion and guidance?	Section 1.2 explains the procedural steps that EFSA has taken following the receipt of the mandate from the European Commission (section 1.1). This section is usually included in most of the scientific opinions produced by EFSA on mandates received by EC.	44
EuropaBio	1.2 Background as provided by EFSA	Lines 101-103: EuropaBio welcomes the opportunity to comment on this document and appreciates the extended deadlines requested by EFSA to the EC.	The GMO Panel thanks for the comment.	45
ENSSER	1.3 Terms of reference	We would like to understand where in the ToR it says that SDNs should be compared to Conventional Breeding. Please provide clarification. Line 110: "may be applicable, in whole or in part…" Does the ToR not also ask if there is more needed? I.e. if it is sufficient? As there are clearly different technical possibilities now than there were then. And SDN3 has a very different purpose than SDN1&2.	The EFSA opinion on SDN-3 was developed by comparing the type of outcome and mutations produced by SDN-3 to those generated by conventional breeding, including random mutagenesis. For this reason, the GMO Panel followed the same approach for SDN-1, SDN-2, and ODM, in order to be able to assess the applicability of section 4 and conclusions of that opinion to plant developed via these approaches. Regarding comment for line 110, each section related to the assessment of the applicability of section 4 and conclusions of the opinion of SDN-3 includes a sub-conclusion indicating whether that section is applicable in whole or in part based on the rationale described before.	46

EuropaBio	1.3 Terms of reference	Lines 113-118: EuropaBio welcomes the opportunity to comment on the draft EFSA opinion on the applicability of the EFSA scientific opinion addressing the safety assessment of plants developed using SDN type 3 to plants developed using SDN type 1 and 2 and ODM. We suggest that here EFSA should stress the importance of the principle of proportionality as set out in Article 5 of Regulation (EC) No178/2002.	The GMO Panel thanks EuropaBio for the comment. This section deals only with the the procedural steps that EFSA has taken following the receipt of the mandate from the European Commission (section 1.1) and should not include any opinion on how the risk assessment should be performed.	47
Association Française de Biotechnologies Végétales	1.3 Terms of reference	AFBV edits and comments: Line 117: replace "or in part, to plants developed", by "or in part, for plants developed". Comment on the scope of technologies: This is the first request by the Commission for an opinion by EFSA on genome editing technologies since 2012. The Commission has asked two narrow questions limited to plants developed with type 1 and type 2 Site-Directed Nucleases and with oligonucleotide directed mutagenesis. Recognizing the rapid evolution of the field of genome editing technologies, EFSA has rightfully chosen to add base editing and prime editing to the scope of its opinion. EU stakeholders would benefit if EFSA were to broaden its opinion to the entire field of genome edit	The GMO Panel thanks for the comments on the terms or reference. It should be noted that this section includes text as it has been provided by the European Commission. A footnote has been added in order to clarify this point. The GMO Panel recognizes that the field of genome editing is rapidly evolving. However, the panel has developed this opinion by strictly adhering to the terms of reference.	48
Euroseeds	1.3 Terms of reference	Considering the importance of the topic, Euroseeds asks the EFSA GMO Panel to broaden its view to the principle of proportionality and give it a more prominent place in the evaluation. The principle of proportionality is set out in Article 5 of the EU Treaty (TEU)[1], and has been included in the general food law which states "In accordance with the principle of proportionality as set out in Article 5 of the Treaty, this Regulation does not go beyond what is necessary in order to achieve the objectives pursued" (Regulation (EC) No 178/2002) and thus it is a principle that needs to be kept in mind in the evaluation of every mandate. In accordance with the principle of proportionality, EFSA would be expected to ensure that its measures and requests are appropriate and non-discriminatory to achieve the overall objective of safety, and do not go beyond what is necessary to achieve that goal.	The GMO Panel takes note of the comment. The panel performs the risk assessment of GMOs according to the all the provisions laid down in the EU regulation of GMOs. The GMO Panel has developed this opinion by strictly adhering to the terms of reference.	49
National Food Institute, Technical University of Denmark	1.3 Terms of reference	The terms of reference is exclusively to compare with the SDN-3 opinion. This opinion on SDN-1 and SDN-2 would however greatly have benefited from a direct comparison with conventional mutagenesis as well.	The EFSA opinion on SDN-3 was developed by comparing the type of outcome and mutations produced by SDN-3 to those generated by conventional breeding, including random mutagenesis. For this reason, the GMO Panel followed the same approach for SDN-1, SDN-2, and ODM, in order to be able to assess the applicability of section 4 and conclusions of that opinion to	50

			plant developed via these approaches.	
German Plant Breeders' Association (BDP - Bundesverband Deutscher Pflanzenzuechter e.V.)	1.3 Terms of reference	BDP acknowledges that the Terms of References for the mandate provided only limited possibility for EFSA to elaborate on the posed questions from a broader perspective taking proportionality into account. However, BDP encourages and requests to take the principle of proportionality into account as it is set out in Article 5 of the EU Treaty (TEU)[1]. It has been considered in European legislation (e.g. European food law, Regulation (EC) No 178/2002) and is a principle that needs to be kept in mind in the evaluation of every mandate. In Regulation (EC) 178/2002 the reference to the principle of proportionality is phrased such that the "Regulation does not go beyond what is necessary in order to achieve the objectives pursued". Accordingly and taking the principle of proportionality into account, EFSA would need to ensure that its measures and requests are appropriate and non-discriminatory to achieve the overall objective of safety, and do not go beyond what is necessary to achieve that goal.	The GMO Panel takes note of the comment. The panel performs the risk assessment of GMOs according to the all the provisions laid down in the EU regulation of GMOs. The GMO Panel has developed this opinion by strictly adhering to the terms of reference.	51
COST Action CA18111 - Plant genome editing – a technology with transformative potential (PlantEd)	1.3 Terms of reference	Regarding Terms of Reference 1 (ToR1), PlantEd notes the following: After the Judgment of the CJEU in case C-528/16, in line with the GMO Panel (that "did not identify any additional hazard associated to the use of the SDN-1, SDN-2 and ODM approaches as compared to both SDN-3 and conventional breeding techniques, including conventional mutagenesis" (EFSA, 2020: 12)), EFSA (2012) "is only partially applicable to SDN-1, SDN-2, and ODM, and may from a strictly scientific perspective not be relevant to the same"; Therefore, PlantEd encourages EFSA to remain as consistent as possible in its scientific approach, while acknowledging the dilemmas that may result from the implementation of recent legal interpretations such as that of the CJEU in case C-528/16. PlantEd invites the EFSA GMO Panel to exercise the proportionality test (a general principle of EU law) in a wider way that reflects more the reality of balancing the different (potential) impacts and to place greater emphasis on case-by-case evaluation. PlantEd considers EFSA's mandate to ensure that all measures adopted do not go beyond what is necessary to achieve the goal stipulated by its mandate.	The GMO Panel takes note of the comment. The panel performs the risk assessment of GMOs according to the all the provisions laid down in the EU regulation of GMOs. The GMO Panel has developed this opinion by strictly adhering to the terms of reference.	52
French agency for Food, Environmental and Occupational Health & Safety (Anses)	1.3 Terms of reference	Page 4, line 117: "are valid […] to plants": "are valid […] for plants" ?	The GMO Panel thanks for the comments on the terms or reference. It should be noted that this section includes text as it has been provided by the European Commission. A footnote has been added in order to clarify this point.	53
Corteva Agriscience	1.3 Terms of reference	As indicated in our comment to the Abstract this mandate is framed in relation to the general food law (Regulation (EC) No 178/2002) and therefore needs to consider the principle of proportionality (which is one of the principles required to be followed by the general food law) in addressing the posed question.	The GMO Panel takes note of the comment. The panel performs the risk assessment of GMOs according to the all the provisions laid down in the EU regulation of GMOs.	54
International Seed Federation	1.3 Terms of reference	ISF believes that the two questions which the GMO Panel was asked to answer gave only a very narrow room for the interpretation of the valuable scientific data was gathered in the report. The	The GMO Panel takes note of the comment. The panel performs the	55

		conclusions could have been more far-reaching if the GMO Panel hadn't been tied to make comparison with their previous opinion on SDN3 and existing legislative framework governing this area but they had been given the possibility to assess the safety aspects of SDN-1, SDN-2 and ODM on their own. ISF would like to ask EFSA to interpret the mandate in a broader manner to include all relevant aspects that help to better reflect on the appropriate and proportionate measures to assess the safety of respective products. Being an international organization ISF has an overview of the policy approaches managing this area globally. Many governments already put in place regulations that are scientifically sound, evidence-based and proportionate to the risks presented by novel characteristics obtained by certain genome editing applications. Examples include Argentina, Chile, Japan, US, Australia etc. Based on these principles countries either excluded certain applications of genome-editing from the scope of their GMO regulations or applied product based consultation procedures to determine the regulatory oversight. None of these approaches requires a specific safety assessment for products obtained by certain genome editing applications. ISF recommends that EFSA should also consider examining the practices of other countries.	risk assessment of GMOs according to the all the provisions laid down in the EU regulation of GMOs. The GMO Panel has developed this opinion by strictly adhering to the terms of reference.	
GenØk-centre for biosafety	1.3 Terms of reference	For details regarding this section: please read our attached table with our comments. **Copied from the submitted pdf file:** EC asks about the safety of certain nucleases not their final product There is a fundamental difference in analyzing the safety of a technique and the safety of a product. In this ToR, EC clearly requests advice on the nucleases and not about the outcome. In this regard, EFSA should provide information on how these nucleases work, their activities and functionalities, the techniques that apply such nucleases, etc. On the contrary, EFSA has only focused on a few intended outcomes of such nucleases. I will provide evidence of such narrow approach in the following sections.	The GMO Panel takes note of the comment.	56
ENSSER	2.0 Data and Methodologies	L126: We would like to understand which criteria have been used to select the specific scientific literature and the "relevant" information, as we find there to be a bias. We miss a horizon scan and a section on risk research, including literature and references to risk research. We miss an update for SDN-3 and the relevant data. Without this the current exercise of comparison is somewhat meaningless. We find a lot of the literature quoted needs to supplemented with more recent literature and research.	The GMO Panel was not mandated to provide an extensive literature review or a horizon scan on SDN-1, SDN-2, and ODM methods. Some references have been added to the text in the relevant sections.	57
French agency for Food, Environmental and Occupational Health & Safety (Anses)	2.0 Data and Methodologies	Page 4, lines 120-123: Proposal to suppress the sentence "EFSA assigned the development of the scientific opinion [...] to the molecular characterisation (MC) working group (WG) of the GMO Panel.", because this is already mentioned in lines 99-101. Page 4, lines 125-126: The reference "EFSA GMO Panel (2012a)" is the same as the one given in the footnote number 3 (page 3). For better clarity, it would be better to move the references that are in the footnotes to the section "6 Reference" (which should be written in the plural form, see comment on line 460) and to cite them accordingly in the text.	Regarding the comment for line 120-123, the text has been removed accordingly. Regarding the comment for line 125-126, the text has been amended accordingly.	58

		Page 4, line 126: "(hereafter, "EFSA opinion on SDN-3")": this could be placed earlier in the text, for instance in lines 81-82 where the opinion is first mentioned, to avoid the repetition of the full title and facilitate the reading.	Regarding the comment for line 126, the text has been amended accordingly.	
ENSSER	2.1.1 Background information	L131: The term NPBTs is not helpful here and should be removed, as it is no longer used and is also implicated in different discussion. The term was not used in EFSA 2012 opinion on SDN3, nor…cisgenesis, except for the appearance of the title in the reference Lusser et al. 2011. L144: Please explain why the comparison is with "conventional breeding" if it has not explicitly been asked in the ToR and also not explicitly and directly been compared in experimentation, and more importantly, why within conventional breeding is the focus on mutagenesis approach? Concerning footnote 5: This definition excludes to our understanding mutational breeding from the definition of conventional breeding as mutations are legally defined as GMOs as per 2001 directive. We would agree with such an interpretation, but it would block the further comparison with mutational breeding. If we are misreading the footnote, please clarify with an legal opinion.	Regarding the comment for line 131, the term "NPBTs" has been removed. Regarding the comment for line 144, the EFSA scientific opinion on SDN-3 was developed by comparing the type of outcome and mutations produced by SDN-3 to those generated by conventional breeding, including random mutagenesis. For this reason, the GMO Panel followed the same approach for SDN-1, SDN-2, and ODM, in order to be able to assess the applicability of section 4 and conclusions of the opinion on SDN-3 to plant developed via these approaches. Please note that a footnote has been inserted in the text to refer to the list of techniques relevant for a comparison as indicated in the opinion on SDN-3 (section 3.2.1). Regarding the comment for footnote 5, the text has been amended both in the footnote and in the main text to improve clarity (end of section 2.1.1).	59
Logos Environmental	2.1.1 Background information	To assert (in footnote 5) that conventional breeding is simply an absence of being covered by EU GMO regulations is not correct, not was it in 2012. The document continually makes reference to mutagenesis techniques as a conventional breeding comparator to genome editing (also in 3.2.2.2.2). This is not correct. Mutagenesis results in GMO plants that are exempt from the EU GMO legislation because of a "history of safe use". Genome editing does not have this history of safe use and is a wholly different suite of techniques to mutagenesis, making the comparison invalid. Importantly, the extent to which genome editing creates unintended genomic alterations is not yet wholly clear, although publications are accumulating showing these unintended effects can be far reaching. It may be several years until the true and complete nature of unintended genomic alterations caused by genome editing becomes clear. These aspects should be reflected in the Opinion.	The EFSA scientific opinion on SDN-3 was developed by comparing the type of outcome and mutations produced by SDN-3 to those generated by conventional breeding, including random mutagenesis. For this reason, the GMO Panel followed the same approach for SDN-1, SDN-2, and ODM, in order to be able to assess the applicability of section 4 and conclusions of the	60

			opinion on SDN-3 to plant developed via these approaches. Please note that a footnote has been inserted in the text to refer to the list of techniques relevant for a comparison as indicated in the opinion on SDN-3 (section 3.2.1). Regarding the footnote 5, please note that the text has been amended both in the footnote and in the main text to improve clarity (end of section 2.1.1).	
Association Française de Biotechnologies Végétales	2.1.1 Background information	AFBV edits and comments: • Line 132: After "by (EFSA GMO Panel, 2012a)," insert "referred to herein as the SDN-3 Scientific Opinion". • Footnote #5: After the first sentence strike the reference to the authors and the title of the SDN-3 opinion (unnecessary) and provide instead the page reference for the first sentence (p. 13). As follows: Conventional plant breeding is defined as methods used by plant breeders for the improvement of commercial varieties and where the resulting plants/varieties are not covered by the legal definitions of genetic modification (in SDN-3 Scientific Opinion at p. 13). (Directive 2001/18/EC) ARPAIA, S., BIRCH, A. N. E., CHESSON, A., DU JARDIN, P., GATHMANN, A., GROPP, J., HERMAN, L., HOEN-SORTEBERG, H. G., JONES, H., KISS, J., KLETER, G., LAGIOU, P., LOVIK, M., MESSEAN, A., NAEGELI, H., NIELSEN, K. M., OVESNA, J., PERRY, J., ROSTOKS, N., TEBBE, C. & MODIFIED, E. P. G. 2012. Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function. EFSA Journal, 10. Immediately thereafter add the following: "In the SDN-3 Scientific Opinion the EFSA GMO Panel considered the following techniques relevant for a comparison with plants developed by the SDN-3 technique: sexual crosses, bridge crosses, embryo rescue, somatic hybridisation, translocation breeding and mutation breeding. SDN-3 Scientific Opinion at p. 14. Under the term "Mutation breeding", the EFSA GMO Panel included spontaneous mutations, induced mutations (chemical and physical mutagenesis) and somaclonal variation. SDN-3 Scientific Opinion at pp. 15-17." We note that the techniques in question were selected by EFSA because they were appropriate comparators; they do not necessarily include all conventional breeding techniques currently available to breeders.	Regarding comment for line 132, the citation of the EFSA scientific opinion on SDN-3 has been revised throughout the text of the opinion to improve consistency. Regarding the comment for footnote 5, please note that the text has been amended both in the footnote and in the main text to improve clarity (end of section 2.1.1).	61
Euroseeds	2.1.1 Background information	Line 144/145: We agree with EFSA's reference to the definition of conventional breeding in EFSA's Scientific Opinion on SDN-3 from 2012 (1) and would like to highlight that not only the definition as quoted in line 144/145 are relevant to understand the concept of conventional breeding but also all the details as laid out in chapter "3. CONVENTIONAL PLANT BREEDING TECHNIQUES RELEVANT FOR A COMPARISON WITH SDN-3 TECHNIQUE". We ask EFSA to specify this. (1) EFSA Journal 2012;10(10):2943	The GMO Panel thanks for the comment. The text at the end of section 2.1.1 has been amended to improve clarity.	62

European Coordination Via Campesina	2.1.1 Background information	To assert (in footnote 5) that conventional breeding is simply an absence of being covered by EU GMO regulations is not correct, not was it in 2012. The document continually makes reference to mutagenesis techniques as a conventional breeding comparator to genome editing (also in 3.2.2.2.2). This is not legally correct. Mutagenesis in vivo results in GMO plants that are exempt from the EU GMO legislation because of a "history of safe use". Genome editing and mutagenesis in vitro does not have this history of safe use and is a wholly different suite of techniques to in vivo mutagenesis, making the comparison invalid. Importantly, the extent to which genome editing creates unintended genomic alterations is not yet wholly clear, although publications are accumulating showing these unintended effects can be far reaching. It may be several years until the true and complete nature of unintended genomic alterations caused by genome editing becomes clear. These aspects should be reflected in the Opinion.	The EFSA scientific opinion on SDN-3 was developed by comparing the type of outcome and mutations produced by SDN-3 to those generated by conventional breeding, including random mutagenesis. For this reason, the GMO Panel followed the same approach for SDN-1, SDN-2, and ODM, in order to be able to assess the applicability of section 4 and conclusions of the opinion on SDN-3 to plant developed via these approaches. Please note that a footnote has been inserted in the text to refer to the list of techniques relevant for a comparison as indicated in the opinion on SDN-3 (section 3.2.1). Regarding the footnote 5, please note that the text has been amended both in the footnote and in the main text to improve clarity (end of section 2.1.1).	63
French agency for Food, Environmental and Occupational Health & Safety (Anses)	2.1.1 Background information	Page 4, line 137: Please write "plant generated…" in the plural form ("plants generated…"). Page 4, lines 139-142: The expression "the Implementing Regulation (EU) No 503/2013 [...] integrated the guidance's requirements in a legal frame." is misleading, because on some topics (e.g. the 90-day feeding study in rodents), the Implementing Regulation (EU) No 503/2013 goes further than the Guidance for risk assessment of food and feed from genetically modified plants (EFSA GMO Panel, 2011). It is therefore proposed to suppress "which integrated the guidance's requirements in a legal frame" at the end of the sentence (new proposal: "It should be noted that the guidance on food and feed risk assessment (EFSA GMO Panel, 2011) was superseded by the Implementing Regulation (EU) No 503/2013."). Page 5, line 143: The use of "To address the requests of the mandate" here is not very clear: is it about the mandate on SDN-3 or about the present mandate on SDN-1, SDN-2 and ODM? It is therefore proposed to replace "To develop the scientific opinion on SDN-3" in lines 132-133 by "Regarding the scientific opinion on SDN-3", and to replace "To address the requests of the mandate, in the EFSA opinion on SDN-3 the GMO Panel compared..." in line 143 by "To develop the scientific opinion on SDN-3, the GMO Panel compared...". Page 5, line 144: The footnote number 5 should be placed earlier in the text, for example in lines 84-85, where the expression "conventional plant breeding techniques" is first used.	Regarding comment for line 137, the text has been amended accordingly. Regarding comment for line 139-142, the sentence has been modified accordingly. Regarding the comment to line 143, the text has been amended accordingly. Regarding the comment to line 144 and the footnote 5, please note that the text has been amended both in the footnote and in the main text to improve clarity (end of section 2.1.1).	64

Corteva Agriscience	2.1.1 Background information	As indicated in our comment to the Abstract this mandate is framed in relation to the general food law (Regulation (EC) No 178/2002) and therefore needs to consider the principle of proportionality (which is one of the principles required to be followed by the general food law) in addressing the posed question.	The GMO Panel takes note of the comment.	65
European Plant Science Organisation, EPSO	2.1.1 Background information	Line 86: Consider further clarifying what is meant by "certain breeding techniques" by rewriting the sentence on line 89 as follows: "...the EFSA GMO Panel considered in vivo and in vitro mutation breeding techniques that emerged before the..."	The GMO Panel notes that the text in section 1.1 was provided by the European Commission as part of the mandate's documentation. A footnote has been inserted to clarify this aspect.	66
Nature et Progrès Belgique	2.1.1 Background information	To assert (in footnote 5) that conventional breeding is Simply and absence of being coveered by EU GMO regulations is not correct, not was it in 2012. The document continually makes reference to mutagenesis techniques as a conventional breeding comparator to genome editing (also in 3.2.2.2.2). This si not correct. Mutagenesis results in GMO plants that are exempt from the EU legislation because of a "history of safe use". Genome editing does no have this history of safe use and is a wholly different suite of techniques to mutagenesis, making the comparison invalid. Importantly, the extent to which genome editing creates unintended genomic alterations is not yet wholly clear, although publications are accumulating showing these unintended effects can be far reaching. It may be several years until the true and complete nature of unintended genomic alterations caused by genome Editing becomes clear. Thse aspects should be reflected in the Opinion.	The EFSA scientific opinion on SDN-3 was developed by comparing the type of outcome and mutations produced by SDN-3 to those generated by conventional breeding, including random mutagenesis. For this reason, the GMO Panel followed the same approach for SDN-1, SDN-2, and ODM, in order to be able to assess the applicability of section 4 and conclusions of the opinion on SDN-3 to plant developed via these approaches. Please note that a footnote has been inserted in the text to refer to the list of techniques relevant for a comparison as indicated in the opinion on SDN-3 (section 3.2.1). Regarding the footnote 5, please note that the text has been amended both in the footnote and in the main text to improve clarity (end of section 2.1.1).	67
Haut Conseil des biotechnologies (High Council for Biotechnology)	2.1.1 Background information	l. 144, footnote 5. As mentioned in l. 84-85, we suggest modifying the definition of conventional breeding techniques as follows: "Conventional plant breeding is defined as methods used by plant breeders for the improvement of commercial varieties and where the resulting plants/varieties do not fall within the scope of Directive 2001/18/EC, either because they do not fall under the legal definition for a GMO, or because they do but they are exempted from application of the Directive".	The EFSA scientific opinion on SDN-3 was developed by comparing the type of outcome and mutations produced by SDN-3 to those generated by conventional breeding, including random mutagenesis. For this reason, the GMO Panel followed the same approach for SDN-1, SDN-2, and ODM, in order to be	68

			able to assess the applicability of section 4 and conclusions of the opinion on SDN-3 to plant developed via these approaches. Please note that a footnote has been inserted in the text to refer to the list of techniques relevant for a comparison as indicated in the opinion on SDN-3 (section 3.2.1). Regarding the footnote 5, please note that the text has been amended both in the footnote and in the main text to improve clarity (end of section 2.1.1).	
Testbiotech	2.1.1 Background information	[line 144, Footnote 5. in the footnote please add:] "For that purpose, conventional breeding was defined as methods ..." [line 159: the reference Andersson et al., 2012 is missing, please add]	Regarding the comment for line 144 and the footnote 5, please note that the text has been amended both in the footnote and in the main text to improve clarity (end of section 2.1.1). Regarding the comment for line 159, the missing reference has been added.	69
GenØk-centre for biosafety	2.1.1 Background information	For details regarding this section: please read our attached table with our comments. **Copied from the submitted pdf file:** Comparison of techniques not adequate EFSA has focused its assessment on the comparison of plants developed using SDN1 and SDN2 to mutagenesis approaches. It is unclear why EFSA has focused on mutagenesis approaches and not addressed the safety of the new nucleases as it stands. If the comparison was made with the intention to have a standard and known technique, EFSA should have focused on techniques that are not genetic engineering techniques as per EFSA Guidance on selection of comparators for the risk assessment of genetically modified plants and derived food and feed2. In addition, mutagenesis approaches can be many techniques with different applications and outcomes. It is not clear, at any part of this document what techniques have been considered, its characteristics and its safety. On top, EFSA defines mutagenesis techniques as conventional breeding techniques: "[…] plants obtained by conventional breeding techniques focusing mainly on mutagenesis approaches." As discussed in the previous section, not only this is a wrong concept and definition but it is vague and does not help with the assessment of a new technique. It does not provide a basis or standard for comparison. It only misleads the assessment as it is not possible to understand at any point of the document to what technique SDN1 and SDN2 are being compared to.	The EFSA scientific opinion on SDN-3 was developed by comparing the type of outcome and mutations produced by SDN-3 to those generated by conventional breeding, including random mutagenesis. For this reason, the GMO Panel followed the same approach for SDN-1, SDN-2, and ODM, in order to be able to assess the applicability of section 4 and conclusions of the opinion on SDN-3 to plant developed via these approaches. Please note that a footnote has been inserted in the text to refer to the list of techniques relevant for a comparison as indicated in the opinion on SDN-3 (section 3.2.1). Please note that the text has been amended both in the	70

			footnotes and in the main text to improve clarity on the comparison between SDN-based methods and conventional breeding approaches (end of section 2.1.1).	
Envirnonmental association Za Zemiata	2.1.1 Backgro und information	To assert (in footnote 5) that conventional breeding is simply an absence of being covered by EU GMO regulations is not correct, not was it in 2012. The document continually makes reference to mutagenesis techniques as a conventional breeding comparator to genome editing (also in 3.2.2.2.2). This is not correct. Mutagenesis results in GMO plants that are exempt from the EU GMO legislation because of a "history of safe use". Genome editing does not have this history of safe use and is a wholly different suite of techniques to mutagenesis, making the comparison invalid. Importantly, the extent to which genome editing creates unintended genomic alterations is not yet wholly clear, although publications are accumulating showing these unintended effects can be far reaching. It may be several years until the true and complete nature of unintended genomic alterations caused by genome editing becomes clear. These aspects should be reflected in the Opinion. [line 144, Footnote 5. in the footnote please add:] "For that purpose, conventional breeding was defined as methods ..." [line 159: the reference Andersson et al., 2012 is missing, please add]	The EFSA scientific opinion on SDN-3 was developed by comparing the type of outcome and mutations produced by SDN-3 to those generated by conventional breeding, including random mutagenesis. For this reason, the GMO Panel followed the same approach for SDN-1, SDN-2, and ODM, in order to be able to assess the applicability of section 4 and conclusions of the opinion on SDN-3 to plant developed via these approaches. Please note that a footnote has been inserted in the text to refer to the list of techniques relevant for a comparison as indicated in the opinion on SDN-3 (section 3.2.1). Regarding the footnote 5, please note that the text has been amended both in the footnote and in the main text to improve clarity (end of section 2.1.1). Regarding the comment for line 144 and the footnote 5, please note that the text has been amended both in the footnote and in the main text to improve clarity (end of section 2.1.1). Regarding the comment for line 159, the missing reference has been added.	71
ENSSER	2.1.2 Section 4 of the EFSA	L150: Whilst this is a verbatum quote, and thus can't be changed, we would like to point out that at the time of its writing no reference was made specifically to epigenetic effects and regulatory processes.	Regarding comment to line 150, as stated in the comment, the text is a quote and cannot be changed.	72

	opinion on SDN-3	These are clearly part part of the resulting genetic changes arising from plant breeding techniques. As of now, so much more is known about those processes, in particular epigenetics, and their importance in plant perforance and gene expression - than were in 2012. This could be acknowledged in a footnote, including stating the need of their inclusion in the assessment of impacts of SDNs of all types. Please explain what is meant by "the primary drivers" - the primary drivers of what? L154: Is this sentence implying that if hazards are arising in both approaches then they are safe, or okay? Do they follow the same risk scenario, or risk hypothesis? Have the same likelihoods? And are they indeed the same? L160: It would be good to include the fact, that SDN3 for plants was not in 2012 or largely is also not now an efficient technique. It would have been important to have looked carefully at examples, checked literature, and investigated the impact on the genome and provide some update and horizon scanning).	As mentioned by the contributor, the epigenetic effects might potentially associate with all the types of genetic modifications and thus are not a new type of the risk associated specifically with SDN-1, SDN-2 and ODM technology. Furthermore, when compared to SDN-3, the risk is even lower, as could be well documented using number of evidence-based studies. Regarding comment to line 154, the scope of the opinion is also to identify potentially novel risks associated with the application of SDN-1, SDN-2 and ODM-based technology compared to SDN-3 and conventional breeding. Regarding comment to line 160, the GMO Panel was not mandated to evaluate the SDN-3 efficiency.	
Association Française de Biotechnologies Végétales	2.1.2 Section 4 of the EFSA opinion on SDN-3	AFBV edit and comment: Line 162: EFSA indicates that the integrated gene can be removed by segregation. This is correct but there exist other means to remove this integrated gene such as a molecular excision process. After "segregation" please insert "or molecular excision".	The GMO Panel thanks for the comment. It should be noted that the text is a quote from the EFSA opinion on SDN-3.	73
Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG)	2.1.2 Section 4 of the EFSA opinion on SDN-3	line 162 add after seggreation - or molecular excision	The GMO Panel thanks for the comment. It should be noted that the text is a quote from the EFSA opinion on SDN-3.	74
French agency for Food, Environmental and Occupational Health & Safety (Anses)	2.1.2 Section 4 of the EFSA opinion on SDN-3	Page 5, line 159: It seems there is a mistake in the reference "(Andersson et al., 2012)". Page 5, lines 160-163: "The SDN-3 technique makes use of the same transformation techniques as transgenesis, although both transient and stable expression of the SDN can be used to introduce the site-specific DSB. In the case of stable integration of the SDN genes, they can subsequently be removed by segregation to obtain plants containing only the integrated gene": 1) This doesn't take into account the cases where the nuclease activity is introduced as mRNA or directly as protein. 2) The transient expression of SDN will have to be demonstrated, as well as the removal of the SDN genes by segregation in case of their stable integration.	Regarding comment to line 159, the reference has been amended accordingly. Regarding comment to lines 160-163, it should be noted that the text is a quote from the EFSA opinion on SDN-3. The abbreviation DSB has been explicated in the text.	75

		3) The abbreviation DSB has not yet been defined.		
European Plant Science Organisation, EPSO	2.1.2 Section 4 of the EFSA opinion on SDN-3	Line 159: Andersson et al. 2012 is not in the list of references. It should probably be (EFSA GMO panel, 2012b). It would also be beneficial to state the main conclusion from that publication, i.e. that hazards should be evaluated on a case by case scenario irrespectively if the inserted DNA is a transgene, intragene or cisgene.	Regarding comment to line 159, the reference has been amended accordingly.	76
Umweltbundesamt (Environment Agency Austria) on behalf of the Austrian lead Competent Authority, the Federal Ministry of Social Affairs, Health, Care and Consumer Protection.	2.1.2 Section 4 of the EFSA opinion on SDN-3	Line 159: Pls. check the literature reference (Andersson et al. 2012). The sentence refers to review work by EFSA, but is missing from the list of references.	Regarding comment to line 159, the reference has been amended accordingly.	77
BUND e.V. / Friends of the Earth Germany	2.1.2 Section 4 of the EFSA opinion on SDN-3	Additional comment: Still, new findings show that removal of SDN genes or remnants of the transformation process by segregation may not be achieved if there are multiple integration sites of foreign DNA sequences (Michno et al. 2020). This must be taken into account for the recommendations based on section 4 of the EFSA opinion on SDN-3.	The GMO Panel thanks for the comment. it should be noted that the text is a quote from the EFSA opinion on SDN3. However, the suggested citation has been taken into consideration by the GMO Panel in developing the document.	78
ENSSER	2.1.3 Conclusions of the EFSA opinion on SDN-3	We regard that the whole section includes probelematic statements, but as it is a quote, such issues would need to be taken up in the section of assesment and cross-referenced from here. Unfortunately neither is currently the case, but we hope this may be altered after this consultation. L173-5: The entier paragraph is problematic, as no systematic experimentation, empirical data production and analysis are provided on wich to base this statement. Again, if compared to just any level of mutagenesis and any technique, then this is not helpful. Such a statement requires urgently the scientific evidence. Please see our comments regarding "fewer" and "same types" for lines 311, 341, 345-347. L183: If the conclusion is that "on a case-by-case basis lesser amounts of event-specific data may be needed for risk assessment" this will require extra guidance as to when and in which way less is needed – and such "opinion" or rather guidance will need to be based on empirical evidence and robust science, including sufficient sets of data. Given that SDN3 was hardly used in plants at the time of writing the 2012 opinion, it should indeed be updated on the basis of CRISPR-based SDN3 research, experiments and development. It would also be important to consider, that more event-specific data may be needed.	Regarding comment to line 173-175, to develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references.	79

			Regarding comment to line 183, depending on the methods which was used to generate the genome edited plant and the traits characterizing such products, the GMO panel may consider some data requirements not necessary for the risk assessment. For this reason, the "case-by-case" approach as described in the opinion on SDN3 is also applicable to genome edited plants. This position is in line with the conclusions of the opinion stating that the EFSA guidances are sufficient but can be only partially applied for the risk assessment of plants generated by the application of SDN1, SDN2, and ODM methods, especially when a transgene and/or exogenous DNA is not present in the final product.	
Association Française de Biotechnologies Végétales	2.1.3 Conclusions of the EFSA opinion on SDN-3	AFBV edits and comments: Line 165: Rewrite beginning of sentence to read: "In 2012, in its overall conclusions". The text which immediately follows in quotes represents EFSA's conclusions in 2012. Line 170: The text from 2012 cannot be changed but in this sentence it would be preferable to use the term "locus" instead of "region". EFSA's 2012 observation regarding the need "on a case-by-case basis" for "lesser amounts of event-specific data" needed for the "risk assessment of plants developed using the SDN-3 technique…. and therefore a need for flexibility in the data requirements for risk assessments…" Since 2012 EFSA has not provided any guidance on what "lesser amounts of event-specific data" would be required for a plant developed using the SDN-3 technique, in particular with respect to a cisgenic plant, regarding which EFSA concluded that risks and hazards are the same as those of conventionally bred plants (EFSA GMO Panel, 2012b). Moreover as pointed out in Lines 141 and 142 the guidance on food and feed risk assessment (EFSA GMO Panel, 2011) was superseded by Implementing Regulation (EU) No 503/2013 which integrated the guidance's requirements in a legal frame. If the Commission and EFSA agree that there is a need for a flexibility in the data requirement for risk assessments, the provisions of Regulation (EU) No 503/2013 should be revisited in a manner to enable EFSA to update its guidances.	Regarding comment to line 165, the reference to the "EFSA opinion on SDN-3" already informs that the statement is from the year 2012. Regarding comment to line 170, the text is a quote from the EFSA opinion on SDN-3 and indeed cannot be change. The GMO Panel thanks AFBV and takes note of the comment on the requirement flexibility for the risk assessment of genome edited plants. Depending on the methods which was used to generate the genome edited plant and the traits characterizing such products, the GMO panel may consider some data requirements not necessary for the risk assessment. For this reason, the "case-by-case"	80

		AFBV proposal for plants derived from the SDN-3 technique. As explained in our comments under Section 3.2.2.1. below, AFBV sent a proposal to the Commission in February 2020 reflecting the need for flexibility in data requirements for certain categories of plants derived from genome editing, including, but not limited to, plants obtained by SDN-1, SDN-2, SDN-3 techniques. In the case of plants obtained by the SDN-3 technique AFBV proposed that, if the inserted gene is a cisgene, the edited plant produced should be excluded from the GMO legislation. EFSA defines a cisgene as "gene from a crossable –sexually compatible – organism (same species or closely related species)". EFSA GMO Panel 2012b. Such edited plants under the AFBV proposal would be excluded from GMO legislation and subject only to regulations applicable to varieties obtained by traditional breeding techniques (see further comments under Section 3.2.2.1 below).	approach as described in the opinion on SDN-3 is also applicable to genome edited plants. This position is in line with the conclusions of the opinion stating that the EFSA guidances are sufficient but can be only partially applied for the risk assessment of plants generated by the application of SDN-1, SDN-2, and ODM methods, especially when a transgene and/or exogenous DNA is not present in the final product.	
Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG)	2.1.3 Conclusions of the EFSA opinion on SDN-3	WGG agrees to the statement in lines 169 - 172 line 170 replace region by locus WGG agrees to the statement in lines 176 - 178 WGG agrees to the statement in lines 183 - 185. However it would be useful to explain a littel bitr more what is the real meaning of "lesser event specific data."	The GMO Panel thanks WGG for the comments. Regarding comment to line 170, the GMO Panel takes note of the suggestion but would like to remind that the text is a quote from the EFSA opinion on SDN-3 and cannot be change. Regarding comment to lines 183-185, the GMO panel may consider some data requirements not necessary for the risk assessment. For this reason, the "case-by-case" approach as described in the opinion on SDN-3 is also applicable to genome edited plants. This position is in line with the conclusions of the opinion stating that the EFSA guidances are sufficient but can be only partially applied for the risk assessment of plants generated by the application of SDN-1, SDN-2, and ODM methods, especially when a transgene and/or exogenous DNA is not present in the final product.	81
GMO Office, National Institute of Public Health and the	2.1.3 Conclusions of the EFSA	In general the RIVM agrees with the conclusion of the GMO Panel with respect to the applicability of the EFSA opinion on the safety assessment of plants obtained by SDN-3 to plants obtained by SDN-1, SDN-2 and ODM and the need for lesser data.	The GMO Panel thanks RIVM for the comment.	82

Environment (RIVM)	opinion on SDN-3			
French agency for Food, Environmental and Occupational Health & Safety (Anses)	2.1.3 Conclusions of the EFSA opinion on SDN-3	Page 5, lines 169-170: "The main difference between the SDN-3 technique and transgenesis is that the insertion of DNA is targeted to a predefined region of the genome.": another main difference is the transient or stable expression of the SDN, or the introduction of the nuclease activity as mRNA or directly as protein. The associated potential hazards need to be studied. Additionally, the transient expression of SDN or the removal of the SDN genes by segregation in case of their stable integration will have to be demonstrated (see comment on lines 160-163). Page 5, lines 170-172: The sentence "Therefore, the SDN-3 technique can optimise the genomic environment for gene expression and minimise hazards associated with the disruption of genes and/or regulatory elements in the recipient genome." gives a very positive view of the SDN-3 technique. A more neutral formulation should be employed, because what is mentioned here needs a certain level of knowledge about the recipient plant genome, which is not the case for all the plant species on which the SDN-3 technique may be used. Page 5, lines 173-175: Same comment as on lines 89-92. Page 6, lines 179-183: Same comment as on lines 25-27 and 93-95.	Regarding comment to line 169, the GMO Panel considers that all the risks associated with transient/DNA-free delivery or stable expression are the same for both SDN-1, SDN-2 and SDN-3, thus not representing any new risk associated specifically with SDN-1 and SDN-2. Nevertheless, the GMO Panel refers the contributor to the section 3.1 and 3.2 where these aspects are addressed. Regarding comment to lines 170-172, the GMO Panel takes note of the comment but would like to remind that the text is a quote from the EFSA opinion on SDN-3 and cannot be change. Regarding comment to lines 173-175 and 179-183, please refer to responses given for line 89-92 and 25-27/93-95, respectively.	83
Haut Conseil des biotechnologies (High Council for Biotechnology)	2.1.3 Conclusions of the EFSA opinion on SDN-3	Although we understand this text comes from EFSA opinion on SDN-3 and is not open to correction, clarification may be provided in footnotes. l. 174. The terms “the same types” should be explained. Considering the evolution of SDN-3 techniques, it could be interesting to have an update of these conclusions with references from papers published since the EFSA opinion on SDN-3 was written.	Regarding comment to line 174, to develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references.	84

			The GMO Panel was not mandated to review or update the conclusions of the EFSA opinion on SDN-3. However, the GMO Panel evaluated the applicability of the conclusions of the SDN-3 opinion to SDN-1, SDN-2 and ODM taking into consideration relevant up-to-date literature on SDN-based approaches.	
GenØk-centre for biosafety	2.1.3 Conclusions of the EFSA opinion on SDN-3	The EFSA opinion draft states that the SDN-3 technique can induce off target changes but fewer that those that can take place during most other techniques used in mutagenesis and where they do occur the changes will be the same as for those produced by conventional breeding techniques. The delivery is more targeted, and not random, as for previous techniques.	The GMO Panel takes note of the comment.	85
Federal Agency for Nature Conservation	2.1.3 Conclusions of the EFSA opinion on SDN-3	Lines 173-175: When comparing SDN techniques with conventional breeding the draft puts off-target changes in both cases at the same level and compares them in terms of number and type. We do not approve this approach as it disregards that conventional breeding and genome editing take two distinct approaches to achieve a new trait: the first one is mainly phenotype-based and the other mainly genotype-based. Conventional breeding comprises of increasing genetic diversity in a first step and then narrowing it down by selection and backcrossing in a second step. Genome editing tries to achieve a new trait in one step. The idea of on-target and off-target changes applies to SDN interventions, but not to conventional breeding as correctly described in the glossary (lines 446-447). There are further reasons why changes at the molecular level in genome editing should not be equated (or rather confused) with mutations in conventional breeding: (i) Some of the intended molecular changes of SDN interventions (see comment under 3.1.1) can hardly or not at all be achieved by conventional breeding. (ii) Off-target changes of SDN interventions are expected to cumulate in sequences similar to the target sequence and therefore, putatively, in functional genetic elements which increases their potential that they are expressed as off-target effects at the phenotypic level. As a consequence off-target changes in genome editing are less evenly distributed across the genome than natural mutations or mutations derived from physical and chemical mutagenesis.	To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references.	86
Federal Agency for Nature Conservation	3.1 Introduction	Lines 186-273: The development of plants by SDN or ODM interventions comprise a couple of steps including the design of the SDN modules and/or the oligonucleotide, their delivery into the cell, their activity to introduce intended as well as unintended changes (see comment on lines 329-330), cell regeneration and tissue cultivation and possibly backcrossing (unless elite lines are directly edited). All of these steps are relevant for the risk assessment of SDN and ODM interventions since some of them can introduce unintended changes and possible risks while others could minder them. Therefore, the assessment should address all of these steps, based on actual data instead of general assumptions. Also because genome edited plants, and especially those of SDN interventions involving multiplexing, may differ in complexity from conventional GM plants (see comment on chapter 3.1.1), it is relevant to include the entire plant (see e.g. Eckerstorfer et al. 2019 and Agapito-Tenfen et al. 2018) rather than to focus on single target sites or whether the product contains exogenous DNA (see comments on 3.2.1 and 3.2.2.2.2).	This opinion is not a standalone guidance for the risk assessment of plants developed through SDN-1,2 and ODM strategies. On the contrary, and in accordance with the mandate received from the EC, this document analyzes the validity of the conclusions of the GMO Panel Opinion on SDN-3 plants and the suitability of the existing framework to asses GM Plants (IR No 503/2013 and EFSA Guidances on the risk assessment of GM	87

			Plants) to assess plants edited through SDN-1,2 and ODM approaches. Therefore, this opinion concentrates on the potential new risks associated with these techniques. Transformation, regeneration, tissue culture, and other techniques mentioned are common techniques used to produce GM Plants (and in some cases for conventional breeding), and their potential associated risks are already covered in the IR No 503/2013 and existing guidance documents. On the other hand, the appropriate description of the methods used is also a mandatory requirement of the existing IR No 503/2013 and EFSA GMO guidances that is considered in this document also relevant for the assessment of edited plants through SDN1,2 and ODM techniques.	
ENSSER	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	L188: "Definition of gene editing" is a wrong title for this section for a number of reasons. Three points concerning the term "gene editing": a) The term "gene editing" is used three times in the document: in the table of content, in this heading and in the title of one reference. The commonly used and more accurate term is "genome editing", which is being used 21 times in this opinion document, 14 times of which in the titles of references. Please ensure consistency in terminology. b) The ToR did not request (or even mention) to address or define "gene editing" or "genome editing", i.e. the term was not included in the request as far as we can tell. Rather, the main term referred to in the request was SDN (site directed nuclease). c) Most importantly: The heading is wrong, as this section does not define gene or genome editing, but rather SDNs as well as ODM; it also explains the categorisation into SDN1, 2 and 3. Please correct the heading accordingly. L201: "the SDN-3 approach can exploit both NHEJ and HDR to insert a large stretch of DNA in a targeted genomic location." This is an ideal scenario setting, as indeed it has been very difficult to insert large stretches of DNA into specific genomic sequences, as the HDR activity in plants is rather low as compared to NHEJ. To clarify this an additional sentence should be added, to let the reader know to which extent this paragraph and the last sentence reflect the reality of research & developments & applications. If indeed this paragraph is intended to provide as 'defnition' of the SDNs, i.e. a theoretical ideal case scenario, then this needs to be made clear. This would be particularly true for	Regarding comment to line 188, the title has been amended to better reflect the content of the section. Regarding comment to line 201, the sentence reports the information provided in the cited references (EFSA GMO Panel, 2012, Podevin et al., 2013). Regarding comment to line 207, the GMO panel considers the sentence clear enough since it already specifies that the modification is related to "a targeted genomic locus". Regarding comment to line 210, the GMO panel considers the sentence clear enough.	88

		SDN3 and SDN2. L207: the term "random modification" for results of SDN1 (at targeted sites) is confusing to the reader, as "random" is primaritly being used to distinguish SDN1 (&2) against random mutagenesis (chemical and physical). Perhaps a 'random targeted mutation' would cause less confusion. L210: please define what is meant to be an "exogenous DNA template", i.e. is it DNA -any DNA, irrespective of its genetic origin- but that has been added in the procedures and will be present in the plant cell at the time of insertion? Or is it meant to limit the meaning of the term to DNA that is sourced from species other than the target organism (which we believe would be inaccurate and unhelpful in the given context). And does the DNA template require to be located outside any chromosomal location – ie in form of a construct as part of a plasmid- or may it already have been integrated into the chromosomal/plastid/genomic DNA previously, i.e. via genetic engineering transformation processes (trans/cis/intra-genesis)? The glossary entry for exogenous DNA does not clarify this sufficiently.		
Association Française de Biotechnologies Végétales	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	AFBV edit: Lines 209 and 210: To be consistent with the second sentence beginning at Line 156, delete "On the contrary", insert instead "In the case of SDN-3," and rewrite the remainder of the sentence to read "the aim of the SDN-3 approach is to modify insert at the targeted locus a transgene, an intragene or a cisgene by inserting an exogenous DNA template of various lengths (e.g; a transgene)".	The sentence has been modified accordingly.	89
Federal Office of Consumer Protection and Food Safety (BVL), Competent Authority according to Directive 2001/18/EC	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	Line 201: Please add for reasons of consistency: "…in a targeted DSB genomic location…"	Regarding comment to line 201, the sentence reports the information provided in the cited references (EFSA GMO Panel, 2012, Podevin et al., 2013).	90
Julius Kühn-Institut	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	L198: Consider to explicitly name the quality of the „template". We suggest to write "… SDN-2 approach makes use of a homeologous template DNA …". For the template will show minor differences to the target sequence, hence, it is not fully homologous, but homeologous. L207 to 208: the modifications are "in general"(!) small and SDN-2 and ODM induce a substitution of a small target sequence or nucleotide: hence, it should be written "…in a small random modification (SDN-1) or in a small intended substitution (SDN-2 and ODM)…" L209: You need to explain what is meant by "foreign DNA". Consider to use "a transgenic element" instead. It keeps a consistent wording with the following sentence about SDN-3.	Regarding comment to line 198, the GMO Panel considers the text to be sufficiently clear. Besides, the terms homeolog/homeologous have a precise meaning in evolutionary biology which would not appropriate here. Homoeologs are pairs of genes that originated by speciation and were brought back together in the same genome by allopolyploidization [Glover, N. M., et al. (2016). "Homoeologs: What Are They and How Do We Infer Them?" Trends in Plant Science 21(7): 609-621)].	91

			Regarding comment to lines 207-208, the GMO Panel considers the text to be sufficiently clear. Regarding comment to lines 209, the expression "foreign DNA" has been replaced by "exogenous DNA" for consistency.	
Euroseeds	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	Line 227: there are many more examples. It should at least be mentioned that the references are examples only. One very comprehensive compilation of examples including 231 market oriented applications was recently published be the German JKI and the German Ministry of agriculture (https://www.bmel.de/SharedDocs/Downloads/DE/_Landwirtschaft/Gruene-Gentechnik/NMT_Uebersicht-Zier-Nutzpflanzen.pdf;jsessionid=B783EDEF6076081A904B7C4691E43E55.internet2842?__blob=publicationFile&v=3) Line 229-234: The more limited amount of information available for ODM compared to SDN-based technologies is less surprising when the enormous number of researchers working on CRISPR-Cas is taken into account. Nonetheless, the molecular mechanism, technological aspects and applications of ODM technology have been described not only in plants but also in microorganisms, animal and human cells in multiple sources including Sauer et al., 2016 (2); Gocal et al., 2015 (4); Dong et al., 2006 (3); Beetham et al., 1999 (7); Alexeev et al., 1998 (6) and Cole-Strauss et al., 1996 (5) . Line 247: Please include also the recent review by Zhang et al.,2020 (3) which gives a better overview of the most recent developments. In line 253 and Table 1, We suggest to rephrase the sentence to be less restrictive: "This step might not be possible in case of non-sexually propagated crops (for example, for vegetatively propagated crops)" as for a number of vegatatively propagated crops crossings are still possible. Line 270 table: The purpose of this table is unclear to Euroseeds. Is it to check, if exogenous DNA might have been integrated and to define the appropriate problem formulation? Unless EFSA makes clear how this table can be used in the risk assessment, the Yes/No criteria for "Exogenous DNA" might be used in some way as a critical differentiator that gives rise to material differences in the way technologies are regulated. The title of EFSA's Table 1 says it's a summary of delivery methods – so it isn't clear how the "exogenous DNA column" is to be understood. The term summarizes very different types of "exogenous DNA" with no explanation or qualification. It's hard to see what the column communicates. E.g. Oligonucleotides as used in ODM or certain SDN-2 applications have end modifications and therefore cannot be stably maintained in the plant cell, they aren't capable of replication and aren't capable of expression. They are small, chemically synthesized single-stranded molecule specifically designed not to be biologically active in the cell/plant. EFSA rightly states that "SDN-1, SDN-2, and ODM approaches differ from SDN-3 and transgenesis in that they do not result in the insertion of any transgene but rather in the modification of an already existing endogenous sequence". In view of this we consider the source and nature of the exogenous DNA as relevant as well and suggest reframing the term as "foreign DNA".	Regarding comment to line 227, the text has been amended to clarify that the cites references provide only some examples of the application of CRISPR-Cas technology to generate genome edited plants. Regarding comment to lines 229-234, the GMO Panel only acknowledges the fact that the data available for plants developed via ODM-based approaches is more limited compared to the data available for the other genome editing techniques. Regarding comment to line 247, the reference Zhang et al.,2020 has been added. Regarding comment to line 253, an alternative sentence has been included in the text. Regarding Table 1, the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	92

		(2) SAUER, N. J., MOZORUK, J., MILLER, R. B., WARBURG, Z. J., WALKER, K. A., BEETHAM, P. R., SCHOPKE, 566 C. R. & GOCAL, G. F. W. 2016. Oligonucleotide-directed mutagenesis for precision gene 567 editing. Plant Biotechnology Journal, 14, 496-502 (3) DONG, C. , BEETHAM, P. , VINCENT, K. AND SHARP, P. (2006) Oligonucleotide- directed gene repair in wheat using a transient plasmid gene repair assay system. Plant Cell Rep. 25, 457–465. (4) GOCAL, G.F.W. , SCHÖPKE, C. AND BEETHAM, P.R. (2015) Oligo- mediated targeted gene editing In Advances in New Technology for Targeted Modification of Plant Genomes Chapter 5 (Zhang F., Puchta H. and Thomson J.G., eds), pp. 73–90. Berlin, Heidelberg: Springer Verlag. (5) COLE- STRAUSS, A. , YOON, K. , XIANG, Y. , BYRNE, B.C. , RICE, M.C. , GRYN, J. , HOLLOMAN, W.K. et al (1996) Correction of the mutations responsible for sickle cell anemia by an RNA- DNA oligonucleotide. Science, 273, 1386–1389 (6) ALEXEEV, V. AND YOON, K. (1998) Stable and inheritable changes in genotype and phenotype of albino melanocytes induced by an RNA- DNA oligonucleotide. Nat. Biotechnol. 16, 1343–1346. (7) BEETHAM, P.R. , KIPP, P.B. , SAWYCKY, X.L. , ARNTZEN, C.J. AND MAY, G.D. (1999) A tool for functional plant genomics: chimeric RNA/DNA oligonucleotides cause in vivo gene- specific mutations. Proc. Natl Acad. Sci. USA, 96, 8774–8778 (8) CRISPR technology in plant science Yingxiao Zhang, Aimee A. https://www.nature.com/articles/s41477-019-0461-5		
Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG)	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	line 210 in the brackets add after transgene, - cisgene, intrgene	Since SDN-3 method aims at inserting any type of DNA sequence, the text in the brackets has been removed.	93
Union Française des Semenciers	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	-Line 270 table: It isn't clear how the criteria "exogenous DNA" must be understood in Table 1. The term comprises very different types of "exogenous DNA" with no explanation or qualification. For instance, oligonucleotides as used in ODM or certain SDN-2 applications have end modifications and cannot be stably maintained in the plant as they are unable to replicate or express.. They are small single-stranded molecules designed on purpose to be biologically inactive in the plant. According to EFSA statement, "SDN-1, SDN-2, and ODM approaches differ from SDN-3 and transgenesis in that they do not result in the insertion of any transgene but rather in the modification of an already existing endogenous sequence". Consequently, no exogenous DNA is integrated in the final product issued from the existing techniques, e.g. CRISPR-Cas system, TALEN, ZFN, Meganuclease or ODM. Therefore, as the purpose of table 1 is to "summariz[e] delivery methods for the SDN and ODM available in plants" UFS suggests that the last column regarding "exogenous DNA" could be misinterpreted. UFS suggests to add "but not integrated" as suggested in uploaded file.	Regarding Table 1, the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	94
GMO Office, National Institute of Public Health and the Environment (RIVM)	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	Line 207: In this line is stated In general, the application of SDN-1, SDN-2, and ODM methods result either in a random (SDN-1) or in an intended (SDN-2 and ODM) modification of a targeted genomic locus without the insertion of foreign DNA. Comment: The terminology with respect to SDN-1 e.g. random modification of a targeted genomic location creates confusion with respect to word 'random', since the intended mutation is a targeted	It should be noted that the term "random" refers to the change of the nucleotide sequence which is not predictable as explained at the beginning of the section. The GMO Panel considers the text sufficiently clear.	95

		mutation. Suggestion is to rephrase the sentence in that it reads that SDN-1, SDN-2 and ODM results in '(targeted) modification of a predefined genomic locus' or something similar.		
National Food Institute, Technical University of Denmark	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	Also the definition seem to be confusing. In some sentences it is clear that SDN-1 and 2 does not involve insertion of exogenous DNA as if this is the definition of these techniques. However, later it is stated that the presence of foreign DNA are not relevant for the risk assessment of plants developed via SDN-1, SDN-2, and ODM approaches in case the genome of the final product does not contain exogenous DNA. If the definition of SDN-1 and SDN-2 is that foreign are not present then you should not analyse for it when making the risk assessment. This analysis should be done in order to establish whether the technique used or the resulting product is defined as SDN-1 or SDN-2 technique/product. SDN-2 is defined as an approach that makes use of a template DNA, whereas SDN-3 is defined as an approach that introduces foreign DNA. But template DNA is also foreign DNA. It is therefore a general question that needs to be considered, where the line between them exactly is to be found. E.g. the number of base pairs that would separate SDN-1/SDN-2 from SDN-3. Also, it could be imagined that a number of SDN-1 or SDN-2 were successively directed to nearly the same place in the genome. Making the product more resemble the outcome of using SDN-3. Again, there is a need for a clearer definition of the different categories. Otherwise explaining the difficulties to make a clear definition and explaining the overlap between the different techniques would be a valuable contribution to the document. Separation due to whether the DNA is inserted or just used as a template seem to be more of a philosophical question since DNA cannot be separated due to if it is the original string or the new copy. When the regulation used the term "capable of continued propagation" this should be related to the traits (mutations included) meaning the sequences whether a copy from a template or the original template. Otherwise it will not make any sense since for all GMO's not only the original inserted sequence is regulated but also the offspring containing copies	The GMO Panel was not mandated to provide definitions for SDN-1, SDN-2, and ODM. In this document, the GMO Panel provides only a description of the differences between these methods and SDN-3. Although the application of SDN-1, SDN-2 and ODM approaches could result in plants where no exogenous DNA has been introduced, in case a transgene is used to introduce the SDN machinery (e.g. the Cas9 gene) which will be still present in the final product, the transgene will need to be risk assessed according to all the EU provisions laid down for the risk assessment of GMOs and the EFSA guidances. The GMO Panel considers these aspects already explained in the opinion.	96
Austrian Agency for Health and Food Safety (AGES)	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	We would like to ask for a more precise definition of SDN-2 and SDN-3 in Chapter 3.1.1 (lines 188-210), since both techniques make use of template DNA to introduce a predicted modification. The EFSA Draft Opinion fails to clearly outline the criteria by which to distinguish between an SDN-2-type and an SDN-3-type modification. In other words, where ends a modification of an already existing endogenous sequence, and where begins an insertion of a transgenic sequences? We consider it important to clearly outline these criteria, since classification as SDN-2 type would mean that for these gene edited plants the sections 4.1 and 4.2.1 of the EFSA Scientific opinion on Zinc Finger Nuclease and other Site-Directed Nucleases (EFSA 2012) would not be (or only in part) applicable. [EFSA, 2012. Scientific opinion addressing the safety assessment of plants developed using zinc finger nuclease 3 and other site-directed nucleases with similar function. The EFSA Journal 10(10):2943: 1-31.]	SDN-3 approaches intent to introduce a transgene in a pre-defined location of the genome, whereas SDN-2 approaches aim at modifying an endogenous genomic sequence. The purpose is therefore clearly different and in most of the cases the edited plants will be easy to classify. The GMO Panel refers to the opinion on SDN-3 for the operational definition of SDN-1 and SDN-2 techniques. Moreover, although the precise boundaries between the two approaches will have to be legally defined if plants obtained through the two approaches are to be treated differently from a legal point of view, this precise definition is not needed in the framework of this document, as all	97

			possible situations have been covered.	
Plantum - Netherlands seed association	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	SDN1, 2 and ODM is a quickly advancing field of research. Additional literature references may be useful throughout the assesment section for completeness. If needed, we could suggest some.	The GMO Panel thanks for the comment.	98
COST Action CA18111 - Plant genome editing – a technology with transformative potential (PlantEd)	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	L. 253: We suggest to broaden the sentence on the possibility of vegetatively propagated crops crossings, in the following way: "This step is, though possible, not routinely carried out in non-sexually propagated crops (such as vegetatively propagated crops)." Figure on page 8: EFSA is correct in stating that "SDN-1, SDN-2, and ODM approaches differ from SDN-3 and transgenesis in that they do not result in the insertion of any transgene but rather in the modification of an already existing endogenous sequence." However, PlantEd holds that the statement can be strengthened by alluding to the source and nature of the exogenous DNA as relevant as well. The figure also needs a correction: (1) at the top of the middle column: "No if crossed out or removed by molecular excision". Furthermore the indication of "exogenous DNA deployed at any stage during the process" is unclear. E.g. oligonucleotides as used in ODM are small, synthesized molecules with no hereditary function. Indicating that exogenous DNA is necessary is in contradiction with the preceding text (line 263): "In case of ODM, the chemically synthetized oligonucleotide is directly delivered to the plant cell without the need of any stable or transient expression system."	Regarding comment to line 253, the sentence has been modified to improve clarity. Regarding Table 1, the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	99
French agency for Food, Environmental and Occupational Health & Safety (Anses)	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	Page 6, line 209: "foreign DNA": the term "exogenous DNA", which is defined in the glossary, should be preferred (same comment as on lines 22 and 29).	The text has been amended accordingly.	100
Corteva Agriscience	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	Line 202: addition proposed "…since it dies not rely on exogenous nucleases or DSB".	The proposed change has not been inserted in the text. The GMO Panel considers the insertion of the term "DSB" too restrictive since some SDN-based approaches do not generate DSB (for example, base and prime editing).	101
European Plant Science	3.1.1 Definition of gene editing:	Line 198: To further clarify the nature of the DNA template, we suggest the following wording: SDN-2 approach makes use of a short DNA template that only differs in one or a few nucleotides from	The GMO Panel refers to the opinion on SDN-3 for the operational definition of SDN-1	102

Organisation, EPSO	SDN 1, SDN-2, and ODM compared to SDN 3	the target sequence, to introduce a predetermined modification (i.e. intended sequence modification) at the target DSB site… Line 207 to 209: It should be further clarified that the induced modifications, in general, are small, i.e. single nucleotide substitutions or small deletions: … SDN-1, SDN-2, and ODM methods result either in small random (SDN-1) mutations, insertions or deletions, or in minor intended nucleotide substitutions (SDN-2 and ODM) at the targeted genomic locus without the insertion of any recombinant/exogenous DNA.	and SDN-2 techniques. Although the application of SDN-based approaches usually produce "small" nucleotide changes, larger sequence changes (for example, deletions in case of SDN-1 type of intervention) can also happen and are in principle not excluded. For this reason, the GMO Panel considers the general text of the opinion more appropriate.	
Haut Conseil des biotechnologies (High Council for Biotechnology)	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	l. 198. "to introduce a predicted modification": Suggestion to replace "introduce" by "generate" to avoid any confusion with a possible physical insertion. l. 207-210. "In general, the application of SDN-1, SDN-2, and ODM methods result either in a random (SDN-1) or in an intended (SDN-2 and ODM) modification of a targeted genomic locus without the insertion of foreign DNA. On the contrary, the aim of the SDN-3 approach is to modify the targeted locus by inserting an exogenous DNA template of various lengths (e.g. a transgene)." This paragraph should be written with more care and precision to avoid any risk of misunderstanding, especially regarding the words "without the insertion of foreign DNA". First, it should be made clear that the method of delivery of the intended modification is not considered here. This method of delivery may include a stable integration, in the recipient plant's genome, of the molecular components necessary to achieve the genetic mutation (see 3.1.3), which may or may not be crossed out at a later stage in the development of the final product. Second, there is also a possible confusion in the understanding of "without the insertion of foreign DNA", whereby there would be a clear mechanistic distinction between SDN-2 and SDN-3. Could EFSA clarify whether there is a documented difference between the molecular mechanisms involved (1) in the generation of a sequence mutation using a DNA template in the case of SDN-2 and (2) in the generation of an insertion of a larger stretch of DNA in the case of SDN-3? If there is a difference, what length of template or of mutated sequence would trigger an SDN-2 rather than an SDN-3 mechanism? Alternatively, if the difference in designation of SDN-2 and SDN-3 is solely based on the nature of the sequence rather than on mechanistic differences ("foreign" being more important than "insertion" in the phrase "without insertion of foreign DNA"), could this be clarified here? Alternatively, it could be underlined that SDN-2 (as SDN-1 and ODM) modifies the sequence and alters the function of a pre-existing gene or regulatory sequence at a given locus, whereas SDN-3 brings a whole transgene with its own function in a pre-determined locus. The difficulty to distinguish SDN-2 from SDN-3 would be narrowed down to instances where an allele is replaced by a modified one using a template DNA spreading the whole gene.	Regarding comment to line 198, the text has been amended accordingly. Regarding comment to lines 207-210, the EC mandate refers to the opinion on SDN-3 and, for this reason, the GMO Panel refers to the opinion on SDN-3 for the operational definition of SDN-1 and SDN-2 techniques. The GMO Panel was not mandated to provide new definitions for SDN-based techniques. Moreover, delivery methods have been considered in section 3.1.3 and have been taken into account in developing this document.	103
Testbiotech	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	[line 201, add additional text]: "In all cases, the result of interventions with SDN is targeted and not random: SDN applications should be regarded as biological mutagens (Fraser et al., 2020) that can, unlike chemical or physical mutagens, interact in a targeted way with the biological mechanisms in the cell, on the level of the genome and/or epigenome. SDN-2 and SDN-3 applications aim to add new genetic information or to alter genetic information in a predefined way. The aim of SDN-1 applications is to impair a specific genetic function that then cannot be restored. In this context, the result of SDN-1 cannot be considered to be random just because it goes along with non-homologous end joining (NHEJ): if the cell tries to repair the genome to restructure it according to the original	The GMO Panel considers that for the comments related to lines 201, 206, 207-209, 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.	104

		function (see, for example, Brinkman et al., 2018), the application of the nuclease will nevertheless typically result in a cell or an organism in which the original genetic function is deleted. Further, SDN-1 applications (as well as other classes of nucleases) are designed to identify distinct genetic sequences, no matter how many copies are present in the genome. This is especially relevant for plants which have several copies of genes in their genome (see, for example, Sanchez-Leon et al., 2018; Kannan et al., 2018; Duensing et al., 2018). Therefore, the pattern of genetic change will typically be different to genetic changes resulting from random processes. Nevertheless, the final result can be impacted by several factors, such as the delivery of the nuclease into the cell, the specificity of the targeted DNA sequences, the number of copies of the targeted DNA sequence(s), the number of different target sequences in the genome, the specific nuclease and the repair mechanisms in the cell (see point 3.1.2)." [line 206, add additional text]: "Similarly to SDN-2 applications, the aim is to change the genome in a way that distinct new information is added or changed in a predefined way. The result will be dose-dependent (Sauer et al., 2016). If applied successfully, it will result in predefined changes of genetic sequences, including all or several copies of the wild gene type. Therefore, in many cases, the pattern of genetic changes may be different to genetic changes resulting from random processes. Nevertheless, also in case of ODM, the final result can be impacted by several factors, such as the process of delivering the nucleotides, the specificity of the targeted DNA sequences, the number of copies of the targeted DNA gene copies, the number of different targets in the genome and the repair mechanisms in the cells." [line 207-209, exchange sentence:] "In general, SDN-1, SDN-2, and ODM applications result in intended modifications or in impairing of gene functions at predefined genomic loci without the insertion of foreign DNA."		
Umweltbundesamt (Environment Agency Austria) on behalf of the Austrian lead Competent Authority, the Federal Ministry of Social Affairs, Health, Care and Consumer Protection.	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	Line 207-209: Again, the absence of foreign or ectopic DNA from products of SDN-1, SDN-2, and ODM methods cannot be assumed, but has to be demonstrated by risk assessment. The discussion should also reflect the differences between the different types of outcomes of SDN-1, SDN-2, and ODM methods: * The opinion should differentiate firstly between approaches targeted to modify single genomic loci on one hand and approaches to introduce multiple genomic modifications (either multiple sites in single target genes, changes in multiple alleles and/or multiple target genes) simultaneously or approaches to sequentially introduce multiple changes with the aim to create larger overall genetic modifications on the other. The different approaches vary widely regarding their depth of intervention and the resulting scope of biological changes; an issue which is highly relevant with a view to risk assessment (Eckerstorfer et al. 2019). * Secondly different types of genome editing approaches need to be considered according to their specific characteristics, namely SDN-applications targeted to introduce double-strand breaks into genomic DNA, SDN-nickases, SDNs modified for base editing or prime editing and modified SDNs introducing epigenetic changes (for overview see e.g. Tycko et al. 2017). The different systems are known to introduce unintended changes of different type and with different frequencies; again, this is important for the assessment of unintended modifications present in products of genome editing required according to Dir. 2001/18/EC. * Last but not least the risk assessment requirements should take into account whether such	The risk assessment of the introduced DNA or the analysis of the absence of this introduced DNA is considered relevant and it is indeed covered in the opinion (please, refer to sections 3.2.1). Although the simultaneous introduction of multiple genomic modifications is not specifically discussed in the opinion, the GMO Panel considers that all the considerations included in the opinion on SDN-based methods are also applicable to this concept. Moreover, it should also be noted that the simultaneous modification of multiple genomic loci is not specific to SDN/ODM approaches as it can also be achieved by	105

		unintended changes may be removed during later steps of crossbreeding or whether the edited plants would be marketed or released without multiple cycles of crossbreeding, such as edited perennial plants like trees, edited vegetatively propagated plants or edited elite lines of crop plants (Eckerstorfer et al., 2019a). Eckerstorfer, Michael F.; Dolezel, Marion; Heissenberger, Andreas; Miklau, Marianne; Reichenbecher, Wolfram; Steinbrecher, Ricarda A.; Waßmann, Friedrich (2019a): An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs). Frontiers in bioengineering and bio-technology 7, S. 31. DOI: 10.3389/fbioe.2019.00031. Tycko, J., Hess, G. T., Jeng, E. E., Dubreuil, M., and Bassik, M. C. (2017). The expanding CRISPR toolbox. Nat. Methods, Available online at: http://s3-service-broker-live-19ea8b98-4d41-4cb4-be4c-d68f4963b7dd.s3.amazonaws.com/uploads/ckeditor/attachments/7742/CRISPR_poster-WEB.pdf USDA-APHIS (2020): Amendment of 7 CFR Parts 330, 340, and 372, Docket No. APHIS-2018-0034, RIN 0579-AE47	transgenic and conventional breeding approaches. The GMO Panel would also like to remind that the "case-by-case" approach can also be applied to genome edited plants. The GMO Panel knows that a complexity of scenarios is possible due to the application of SDN-based methods. In this regard, the GMO Panel refers to the mandate on GM plant generated via synthetic biology approaches. The risk assessment of potential unintended effects is included in the IR No 503/2013 and EFSA guidances and is considered relevant for the risk assessment of genome edited plants. The fact that back-crossing may help to eliminate some off-target modifications in some cases is also discussed in section 3.2.2.2.2.	
V, Ganesh kumar	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	In Line number 197 of Page 6 it was mentioned that "random mutations (substitutions, insertions, and deletions) at the target DSB site". More clarity is required here about whether all kinds of the mentioned mutation types are allowed (i.e. small Vs Large mutations: for example, small deletion, small insertion, deletion of entire gene, deletion of large chromosomal segments, deletion of gene regulatory regions, large insertions, etc.,). Inversions and duplications (small or large) can also happen during NHEJ and hence it also should be included under SDN-1. In Line number 198 to 200 of Page 6 it was mentioned that "SDN-2 approach makes use of a template DNA to introduce a predicted modification (i.e. intended sequence modification) at the target DSB site by exploiting the plant homology-directed repair (HDR) pathway". More clarity is needed about the classification of intended sequence modifications achieved without the use of template DNA (for example "Base editing" makes intended substitutions without using template DNA); these type of intended modification can be classified as SDN-1.	The precise legal definition of what should be considered as SDN-1 or SDN-2 is outside the scope of this mandate received from the EC. For this reason, the GMO Panel refers to the opinion on SDN-3 for the operational definition of SDN-1 and SDN-2 techniques. However irrespective of what should be legally considered as SDN-1 or SDN-2, the characterization of the target locus is an important mandatory part of the risk assessment. Therefore, the potential risks associated to any modification at the target locus will be assessed and a scientific opinion addressing these risks will be issued for every product carrying such modifications.	106

International Seed Federation	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	SDN-3 includes a new sequence at that particular genomic location, however new insertion can include sequences derived from within or outside the gene pool of the species. The exact meaning of the term "exogenous DNA" requires clarification. We suggest introducing the term "foreign DNA" instead and rephrase the sentence accordingly. This is more in line with the terminology used by the CBD and GMO legislation globally. Since EFSA indicates on several occasions that SDN1-SDN2-ODM plants are free of transgenic sequences, then it is important to clearly identify in what context the term exogenous is used. A better explanation and more context should be provided.	The GMO Panel preferred the use of the term "exogenous DNA" whose definition is provided in the glossary. The text of the opinion has been revised accordingly.	107
Cornell University's Alliance for Science	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	The Panel's comparison between the techniques of SDN3/transgenesis and SDN-1, SDN-2, and ODM clearly demonstrated how the latter techniques could be used to obtain a plant with or without exogenous DNA present in its genome.	The GMO Panel thanks for the comment.	108
GenØk-centre for biosafety	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	For details regarding this section: please read our attached table with our comments. **Copied from the submitted pdf file:** Definition of gene editing is out of the scope of this mandate EFSA should avoid using the term "definition" as it relates to "legal definition" especially since there are current discussions on the topic. In addition, the issue is not in the mandate of EFSA. In fact, according to the latest ECJ ruling on mutagenesis, the definition of gene editing is the same as the GMO definition as per GMO Directive 2001/18/EC. We suggest merging this topic with the following topic and change the title to "Techniques used in SDN-1, SDN-2 and ODM applications".	The title of section 3.1.1 has been changed to address this comment.	109
Federal Agency for Nature Conservation	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	Lines 189-210: The draft does not properly describe the object of investigation. A chapter is missing that describes the potential and the extent of current and conceivable genomic interventions by SDN-1 and SDN-2. This should comprise the editing of several genes or copies of a gene either simultaneously (multiplexing) or consecutively and the possibility for deep genomic interventions, i.e. the editing of genes which are genetically linked or for other reasons hardly accessible through conventional breeding (see examples in Eckerstorfer et al. 2019 and Kawall 2019). It even seems possible now to restructure whole chromosomes (Beying et al. 2020). These possibilities constitute the great promises of SDN interventions, repeatedly highlighted in discussion about the potential of genome editing; it therefore is unjust to exclude them from this draft and not to consider these cases for risk assessment. Beying, N., Schmidt, C., Pacher, M. et al. CRISPR–Cas9-mediated induction of heritable chromosomal translocations in Arabidopsis. Nat. Plants (2020). https://doi.org/10.1038/s41477-020-0663-x Eckerstorfer, Michael F.; Heissenberger, Andreas; Reichenbecher, Wolfram; Steinbrecher, Ricarda A.; Waßmann, Friedrich (2019): An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs). In: Frontiers in bioengineering and biotechnology 7, p. 319. DOI: 10.3389/fbioe.2019.00031. Kawall, Katharina (2019): New Possibilities on the Horizon: Genome Editing Makes the Whole Genome Accessible for Changes. In: Front. Plant Sci. 10, p. 280. DOI: 10.3389/fpls.2019.00525.	The mandate received does not cover an extensive review of the technical aspects and the potential of these technologies. This document discusses the validity of the conclusions of the EFSA opinion on SDN-3 for plants developed through SDN-1, SDN-2 and ODM. The GMO Panel understands that the term "multiplexing" used in the comment may refer to the simultaneous mutation of multiple plant genomic loci. Although multiplexing approach is not specifically discussed in the opinion, the GMO Panel considers that all the considerations included in the opinion on SDN-based methods are also applicable to multiplexing approaches.	110

			Moreover, it should also be noted that multiplexing is not specific to SDN/ODM approaches as it can also be achieved by transgenic and conventional breeding approaches. The GMO Panel would also like to remind that the "case-by-case" approach can also be applied to genome edited plants. The GMO Panel knows that a complexity of scenarios is possible due to the application of SDN-based methods. In this regard, the GMO Panel refers to the mandate on GM plant generated via synthetic biology approaches.	
Envirnonmental association Za Zemiata	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	[line 201, add additional text]: "In all cases, the result of interventions with SDN is targeted and not random: SDN applications should be regarded as biological mutagens (Fraser et al., 2020) that can, unlike chemical or physical mutagens, interact in a targeted way with the biological mechanisms in the cell, on the level of the genome and/or epigenome. SDN-2 and SDN-3 applications aim to add new genetic information or to alter genetic information in a predefined way. The aim of SDN-1 applications is to impair a specific genetic function that then cannot be restored. In this context, the result of SDN-1 cannot be considered to be random just because it goes along with non-homologous end joining (NHEJ): if the cell tries to repair the genome to restructure it according to the original function (see, for example, Brinkman et al., 2018), the application of the nuclease will nevertheless typically result in a cell or an organism in which the original genetic function is deleted. Further, SDN-1 applications (as well as other classes of nucleases) are designed to identify distinct genetic sequences, no matter how many copies are present in the genome. This is especially relevant for plants which have several copies of genes in their genome (see, for example, Sanchez-Leon et al., 2018; Kannan et al., 2018; Duensing et al., 2018). Therefore, the pattern of genetic change will typically be different to genetic changes resulting from random processes. Nevertheless, the final result can be impacted by several factors, such as the delivery of the nuclease into the cell, the specificity of the targeted DNA sequences, the number of copies of the targeted DNA sequence(s), the number of different target sequences in the genome, the specific nuclease and the repair mechanisms in the cell (see point 3.1.2)." [line 206, add additional text]: "Similarly to SDN-2 applications, the aim is to change the genome in a way that distinct new information is added or changed in a predefined way. The result will be dose-dependent (Sauer et al., 2016). If applied successfully, it will result in predefined changes of genetic sequences, including all or several copies of the wild gene type. Therefore, in many cases, the pattern of genetic changes may be different to genetic changes resulting from random processes. Nevertheless, also in case of ODM, the final result can be impacted by several factors, such as the process of delivering the nucleotides, the specificity of the targeted DNA sequences, the number of copies of the targeted DNA gene copies, the number of different targets in the genome and the repair	The GMO Panel considers that for the comments related to lines 201, 206, 207-209, 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.	111

		mechanisms in the cells." [line 207-209, exchange sentence:] "In general, SDN-1, SDN-2, and ODM applications result in intended modifications or in impairing of gene functions at predefined genomic loci without the insertion of foreign DNA."		
BUND e.V. / Friends of the Earth Germany	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	Line 209 ADD after "foreign DNA": ", although DNA sequences from the process of inserting the CRISPR/Cas complex may remain in various genomic integration sites (Michno et al. 2020)". Additional comment: The cited literature shows, that removal of SDN genes or remnants of the transformation process by segregation may not be achieved if there are multiple integration sites of foreign DNA sequences (Michno et al. 2020). This must be taken into account for the recommendations based on section 4 of the EFSA opinion on SDN-3.	The GMO Panel considers that for the comments related to line 209, 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. The GMO Panel would like to remind that the "case-by-case" approach can also be applied to genome edited plants, as indicated in the document, which is in line also with the general conclusions of the EFSA opinion on SDN-3.	112
CropLife Canada	3.1.1 Definition of gene editing: SDN 1, SDN-2, and ODM compared to SDN 3	-Lines 209-210: SDN-3 includes a new sequence at a particular genomic location, and a new insertion can consist of sequences derived from within or outside the gene pool of the species. We are not certain about the exact meaning of the term "exogenous DNA." We suggest introducing the term "foreign DNA" instead and rephrase the sentence accordingly. -Lines 229-230 and Lines 337-238: There seem to be two contradictory statements about ODM's; "ODM technology has been only used to generate GM plants" and "ODM is practically applied only to generate targeted gene modification which resembles those of the SDN-2 type". We support the use of the latter statement.	Regarding comment to lines 209-210, the definition of the term "exogenous DNA" is included in the glossary; the definition is derived from the EU SAM document on "New techniques in agricultural biotechnology" (European Commission, 2017). Regarding comment to lines 229-230, the GMO Panel considers the two statements not to be contradictory. The first statement refers to the fact that ODM technology has only been used in plants, while the second statement refers to the type of genomic modification achievable with this technique (i.e. SDN-2 type).	113
ENSSER	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	L216-18: Whilst the GMO Panel of EFSA was "not requested" to deliver an "extensive" literature review on the technologies deployed – as explicitly stated by the panel in this section – we regard it as wholely insufficient what is being presented here. Given that CRISPR/Cas did not even exist as a technology at the time of the writing of the 2012 opinion, and also given that SDN3 was not -and has not been since- a straight forward and efficient technology, a solid literature review and a horizon scanning exercise would have been essential for the task at hand.	Regarding comment to lines 216-218, the GMO Panel considers the comment out of the scope of the mandate. The GMO Panel was not mandated to provide neither a comprehensive literature review	114

		Especially "considering the advances in genome editing that unfolded in recent years" (line 217/8) it would deem more than appropriate, i.e. essential, to include solid information, in particular information relevant to risk assessment, including detailed information from the literature from the field of risk research. The literature in the opinion as it is presented in the draft is currently biased. Please ammend these shortcomings – both for this section and next one. The current phrasing "to include some literature" gives the impression of randomness and suitable selection, and not a systematic, scientific and broad-viewed approach. Thus criteria should be added as to what selection criteria were/are chosen for literature and its information to be included. It would perhaps be best to include an extra section with a horizon scan, which could build on the work covered in Eckerstorfer et al. (2019) and Modrzejewski et al. (2019) as well as a new section on risk research. Line 224: Please clarify when saying "derived from genome edited soybean" which form of genome editing was used (i.e. ZFN, TALENs, Meganuclease), otherwise the information offered is of little use. Line 226: At this stage we find that at least three complete sections are missing and should be included in order to cover important and so far largely missing aspects important to risks and risk assessment. These sections should include and elaborate on: - detailing that there is not just one CRISPR-associated nuclease, but that due to their individual specifications and limitations there has been a search for further sources of Cas and for further endonucleases (e.g. Cpf1), as well as the further engineering and amending of these molecules. It is a whole field of research in itself, with relevance to risk assessment. It needs to be clarified that the technology is not a fixed one, but rather that it is fluid and in a constant state of alteration, adjustment and improvement, as well as building purpose-specificity. - the capacity of SDNs to achieve complex changes, whether this is by multiplexing, serial applications of SDN-1 and/or SDN-2, by specifically designed SDNs that will not induce DSBs but alter particular basepairs, etc. There are so many new possibilities to change target sites that come with genome editing that it is crucial to adress this here, as it also reflects new sources and quality of hazard. It also gives rise to a risk assessment problem, namely that of a comparator. Furthermore, the constant further development of the techniques and of SDNs – esp. new CRISPR-variants- open up new areas of the genome for modification. - A lot of research went into getting CRISPR/Cas9 and other CRISPR-based SDNs to work in different plant systems, and to increase efficiency both in terms of activity of the nuclease as well as of the specificity of the guide RNA. The goal is not always application and commercialisation but often improvement of methodology and basic research to deepen the understanding of crop plants, their omics and traits. L227: Rice has become a major model plant, this should be said clearly, please adjust sentence to: "..including model plants such Arabidopsis, tobacco and now also rice, with first reports as early as 2013 (Jiang et al., 2013, Li et al., 2013, Nekrasov et al., 2013), and continuing until now ." In fact, rice has become the number one model plant for SDN research, which is illustrated in Eckerstorfer et al. (2019) and Modrzejewski et al. (2019) L227-29: The sentence as it stands gives the impression that most of the work carried out in the crop plants listed, or in crop plants in general, was carried out on "important agronomic traits", and in particular that the work was done to 'enhance' these traits. This is a clear misrepresentation of what actually	nor an horizon scan on the SDN-based technology. Regarding comment to line 224, Calyno™ High Oleic Soybean Oil has been obtained using TALENs approach. The information has been added to the text of the opinion. Regarding comment to line 226, The GMO Panel was not mandated to provide neither a comprehensive literature review nor an horizon scan on the SDN-based technology. For this reason, the GMO Panel considers not to be necessary to include in the opinion the sections proposed in the comment. Regarding comment to line 227, the GMO panel agree that rice is a major model plant but does not consider that this should be emphasized here. Regarding comment to line 227-229, the GMO panel considers that multiple examples exist where the CRISPR-Cas system has been applied in genome editing to achieve important agronomic traits. Regarding comment to line 228-229, more recent references have been added to the text (Modrzejewski et al., 2019; Afzal et al., 2020). Regarding comment to line 235, the GMO Panel was not mandated to provide neither a comprehensive literature review nor an horizon scan on the	

		has been happening. Whilst the argument for crop improvement will consistently be placed into the abstracts and introducion of papers (largely due to funding needs and obligations), most of the research on crop plants has been on methodology and getting the technology to work, as well as increasingly for basic research to understand the role of various genes and their products as well as that of regulatory sequences. Out of 114 publications using CRISPR/(Cas/Cpf) in plants between (Jan 2016 - June2017), 72 were on methodology, 22 on basic research and only 20 on applied development, see table 2, Eckerstorfer et al. (2019). CRISPR clearly is a research tool. And it has been helpful for this, which is being reflected in the number of papers released. How many important agronomic traits have really been "enhanced" and shown to perform well under normal growing conditions cannot be inferred from quantitative listings. This is obviously also not the task of this brief overview, though it may be fair to say that research is being carried out on various major crop plants, often with the stated goal towards improvement, but without actually doing so. Concerning the listing of references provided in lines 228/9 – this list deems seriously outdated. Furthermore, neither of these papers sat out to achieve enhancement of major agronomical traits, but rather to test that the methodology and technique could in principle be used for the purpose of genome editing of the particular crop plants in question, namely wheat and maize in these cases. Please correct your wordings accordingly. And we urge to update the list of literature cited to include recent research. For overviews, please see Eckerstorfer et al. (2019) and Modrzejewski et al. (2019). Line 235: There are two major sections section missing. 1) Where is the data on efficiency, on off-targets effects, on unintended on-target effects? On risk research?? On which method was used to check for off-target modifications and whether these relied on algorithms, on whole genome sequencing and in that which methodology is used (e.g. such as long read next generation sequencing). The lack of this data is a serious omission and needs to be rectified, also allowing another round of public consultation. 2) A section on multiplexing, deep intervention, repeated or serial applications (e.g. Eckerstorfer et al. 2019; Kawall 2019, Kawall et al. 2000). This is an area with serious risk assessment implications, and it is part of SDN1, as well as SDN2. It is strongly overlapping with synthetic biology, complex interventions and metabolic engineering, and cannot be omitted here. Line 236: The use of "random" is problematic here in this context, as it is not quite correct, as it compares itself with random mutagenesis, despite it being targeted and intentional. Line 238: Please make a paragraph break before "Other techniques", as this is a separate new topic, which needs further elaboration. Both "base editing" and "prime editing" need further explanations, including references to risk research. Furthermore, these two techniques are distinctly different from each other, with prime editing being a most recent development – they should not be placed in one sentence with the same claims of capacity or familiarity. It is not prudent to iterate claims here what prime editing can do, but should be reported in a way that shows that it is the devlopers who think or hope what it may be able to do. Line 246/7: Whilst there is need for further review, such review also needs to extend to the area of risk research.	SDN-based technology. For this reason, the GMO Panel considers not to be necessary to include in the opinion the sections proposed in the comment. Regarding comment to line 236, the GMO Panel considers the term "random" correct in this context since the term refers to a random mutation generated in a "targeted genomic locus". Regarding comment to line 238, the GMO Panel was not mandated to provide neither a comprehensive literature review nor an horizon scan on the SDN-based technology. The GMO Panel acknowledged the existence on emerging techniques like base editing and prime editing focusing mainly on the outcome of these technologies which would produce SDN-2 type of mutations. Regarding comment to line 246-247, the list of references already includes, among others, also recent reviews (Chen et al., 2019, Hua et al., 2019).	

		On the whole, the currently selected examples are insufficient, with 2014 also not fitting the criteria of a "recent" review, and 2017 only just about fitting it. For a more comprehensive picture please add the following: Eckerstorfer et al. (2019), Agapito-Tenfen et al. (2018), Kawall (2019) and perhaps Zhang et al. (2018).		
EuropaBio	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	Line 197: Evidence in human cell lines indicates repair is not random, so we would suggest deleting "random" (also in lines 207 and 236). Lines 197-200: As written, this paragraph indicates HDR as the only pathway for template edits in SDN2. It is still feasible for a NHEJ-mediated repair to have the same intended outcome for a predicted/desired SDN2 edit. The distinction then with what is written in the following lines of the same section for SDN3 are the 1) size of the intended edit (which SDN3 only mentions 'a large stretch of DNA' – how large is large?) and 2) defining foreign or exogenous DNA of various lengths (again what length size, and how is foreign or exogenous DNA defined?). If the exogenous DNA introduced is native or cis to the species other than the desired SDN2 edit, then does it matter whether HDR or NEHJ is utilized (and the plant cell repair pathway will be in charge of that anyway)? Lines 226-227: there are multiple references referring to CRISPR-Cas applications in plants. EFSA should clarify that the references cited here are only some examples. Line 241-242: base editing fits the definition of SDN1 better since no template is provided and a DSB break is not required. EuropaBio recommends reflecting this in the opinion accordingly.	Regarding comment to line 197, the GMO Panel considers the term "random" correct in this context since the term refers to a random mutation generated in a "targeted genomic locus". Regarding comment to lines 197-200, the GMO Panel defined SDN-2 and SDN-3 taking into consideration the end product. In this respect, SDN-3 contains "exogenous DNA" at the targeted locus, while SDN-2 doesn't. The GMO panel agrees that one of the challenges concerning the definition of SDN-2 will be to set the level of heterogeneity between the pre-locus and the modified locus that will define an "exogenous DNA". Regarding comment to lines 226-227, the text has been amended accordingly. Regarding comment to lines 241-242, the operational definition of SDN-1, SDN-2, and SDN-3 in this opinion reflects the outcome of the end product rather than the technology used. The outcome of the application of base editing and prime editing can be predicted, as it is the aim of the SDN-2 approach.	115
Association Française de Biotechnologies Végétales	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	AFBV edit and comments: Line 236: Consistently with Line 156, insert ", an intragene or a cisgene " before "(SDN-3)".	Regarding comment to line 236, the words "an intragene or a cisgene" were inserted in line 236 and 293 but not in line 156 because the text in that section is derived from the opinion on	116

		Terms of reference for genome editing techniques. The Commission's question limits the scope of the terms of reference to SDN1, SDN2 and ODM techniques, known and used long before 2012. Realizing the extent of scientific progress during the last 20 years, EFSA chose to extend the scope of the Commission's question to two newly developed techniques: base editing and prime editing. EFSA has suggested that these two techniques correspond to the category SDN-2. It is true that the type of modification is the same as with SDN-2, but no matrix is used in base or prime editing. Since the early 1990's genome editing techniques have been developing rapidly and this trend is continuing. This is why AFBV suggests a different approach: (i) define genome editing (see below) and (2) consider the types of plants resulting from the use of a genome editing technique. AFBV proposes that the conclusions EFSA is already willing to apply to plants resulting from base editing and prime editing be extended to all techniques currently used to perform genome editing. Definition used by AFBV for genome editing: Genome editing brings together a set of technologies allowing the modification of genetic information by addition, deletion or exchange (replacement) of nucleotides at a targeted site of the genome sequence of a recipient plant.	SDN-3. Moreover, the text of the opinion has been modified by replacing the term "transgene" with "DNA sequence". The operational definition of SDN-1, SDN-2, and SDN-3 in this opinion reflects the outcome of the end product rather than the technology used. The outcome of the application of base editing and prime editing can be predicted, as it is the aim of the SDN-2 approach. The GMO panel thanks AFBV for its comment. The GMO panel thanks AFBV for its comment on a proposed definition of genome editing.	
Federal Office of Consumer Protection and Food Safety (BVL), Competent Authority according to Directive 2001/18/EC	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	Line 242: The wording „…that the genetic modifications obtained using base editing and prime editing fall under the SDN-2 definition (see section 3.1.1)" is unfortunate, because the definition used in 3.1.1 implies the occurrence of DNA double strand breaks. This however, is not true for base and prime editing. We suggest the following wording: "…that genetic modifications obtained using base editing and prime editing are comparable to those created by SDN-2 technology."	Regarding comment to line 242, the GMO Panel thanks for the proposed text improvement. The text of the opinion has been amended accordingly.	117
Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG)	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	line 237 add after a transgene - an intragene or a cisgene	Regarding comment to line 237, the text of the opinion has been modified by replacing the term "transgene" by "DNA sequence".	118
French agency for Food, Environmental and Occupational Health & Safety (Anses)	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	Page 6, lines 213-214: Why does the sentence "In addition, a literature review […] was included (section 2.1 of EFSA GMO Panel (2012a))." start with "In addition"? What is additional as compared with what is mentioned in the preceding sentence "The EFSA opinion on SDN-3 addressed the development and the application of technologies in the area of plant genome editing up to the year 2012."? Proposal to suppress "In addition". Page 7, lines 226-229: The sentence "The CRISPR-Cas system has been applied in genome editing across multiple plant species, including model plants [...] but also to enhance important agronomic traits in crops like maize…" doesn't seem correct on a grammatical point of view. Proposal to	Regarding comment to lines 213-214, the GMO Panel thanks for the comment. The expression "in addition" has been replaced by "In this regard,". Regarding comment to lines 226-229, the GMO Panel thanks for the comment. The expression "to	119

		suppress "to enhance important agronomic traits" (new proposal: "The CRISPR-Cas system has been applied in genome editing across multiple plant species, including model plants [...] but also crops like maize…").	enhance important agronomic traits" has been removed.	
Corteva Agriscience	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	We highly appreciate that the EFSA GMO Panel included some information and multiple references on the more recent advances in genome editing. Lines 228-229: We propose to add additional examples demonstrating even broader spectrum of opportunities for food, feed and industrial use crops to be improved through genome editing – such as tomatoes (e.g., good review in 1; also, 2,3), oil crops (e.g., 4-10). 1. Rothen C., Diouf I., and Causse M (2019) Trait discovery and editing in tomato. The Plant J. 97: 73-90. 2. Li. R. et al. (2018) CRISPR/Cas9-mediated mutagenesis of IncRNA1459 alters tomato fruit ripening. The Plant J. 94: 513-524. 3. Ortigosa A. et al. (2019) Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of SIJAZ2. Plant Biotechnology J. 17: 665-673. 4. Haun W. et al. (2014) Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. Plant Biotechnology J. 12: 934-940. 5. Al Amin N. et al. (2019) CRISPR-Cas9 mediated targeted disruption of FAD2-2- microsomal omega-6 desaturase in soybean (Glycine max L.) BMC Biotechnology 19: 9. 6. Zhang P. et al. (2019) Multiplex CRISPR/Cas9-mediated metabolic engineering increases soya bean isoflavone content and resistance t soya bean mosaic virus. Plant Biotechnology J. doi: 10.1111/pbi.13302. 7. Zheng M. et al. (2019) Knockout of two BnaMAX1 homologs by CRISPR-Cas9-targeted mutagenesis improves plant architecture and increases yield in rapeseed (Brassica napus L.). 8. Wang L. et al. (2020) Natural variation and CRISPR/Cas9-mediated mutation in GmPRR37 affect photoperiodic flowering and contribute to regional adaptation of soybean. Plant Biotechnology J. doi: 10.1111/pbi.13346. 9. Jiang W.Z. et al. (2017) Significant enhancement of fatty acid composition in seeds of the allohexaploid, Camelina sativa, using CRISPR/Cas9 gene editing. Plant Biotechnology J. doi: 10.1111/pbi.12663. 10. McGinn M. et al. (2018) Molecular tools enabling pennycress (Thlaspi arvense) as a model plant and oilseed cash cover crop. Plant Biotechnology J. doi:10.1111/pbi.13014. Lines 235-243: In description of the outcomes of SDN-1, SDN-2, ODM, and SDN-3 approaches, we strongly encourage to also give a separate consideration to the allele replacement application. The gene editing mediated allele replacement is the SDN-2 application, by virtue of being an edit of (not	The GMO panel thanks Corteva for its comment. Regarding comment to lines 228-229, the GMO Panel was not mandated to provide a comprehensive literature review on SDN- and ODM-based technologies, including their application to different agro-food sectors. Regarding comment to lines 235-243, the GMO Panel considers that the text of the opinion sufficiently clarifies that SDN-2 covers also SNP modification. Regarding comment to lines 241-243, The GMO Panel would like to clarify that the mandate focuses on the outcome of the applied technology rather than the technology itself. For this reason, no specific technique was highlighted in the Abstract.	120

		an addition to) the existing gene through the HDR mechanism which replaces the existing allele, and with the same outcome as happens through the conventional breeding cross. Lines 241-243: We appreciate calling out the newer techniques (base editing and prime editing) and clarifying that for the purpose of this opinion they were classified as the SDN-2 application of genome editing technology. We propose to reflect this in the Abstract as well, as these lines appear to be the only place in the document where it is brought up.		
European Plant Science Organisation, EPSO	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	Line 242: EPSO agrees with the opinion that base editing and prime editing are considered equal to SDN-2 since these approaches generate intended modifications of similar extent.	The GMO panel thanks EPSO for its comment.	121
Haut Conseil des biotechnologies (High Council for Biotechnology)	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	l. 229-234. It is said that "the amount of information available in the literature" concerning the ODM technology is limited. Could the corresponding few references be cited here? l. 239. We suggest replacing "introduce" by "generate" to avoid any confusion with a possible integration of DNA.	Regarding comment to lines 229-234, the references for studies concerning ODM can be found in Modrzejewski et al., 2019 which has been added to the section. The GMO Panel thanks for the suggestion. The text has been amended by replacing the term "integrate" with the term "generate".	122
Testbiotech	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	[Line 211: Exchange heading]: "Technology used in SDN-1, SDN-2, and ODM applications, its potentials and its restrictions" [line 220: insert subheading:] "3.1.2.1: Overview of current applications" [line 227, after brackets, replace: instead of "...but also to enhance important agronomic traits in crops like.. "]: "… but also to alter relevant traits of important crops like .." [line 235: insert subheading:] "3.1.2.2: Overview of potentials and restrictions" [Line 235-238 insert changed text instead of 'random':] "It should be noted that while ZFNs, TALENs, meganucleases, and CRISPR-Cas system can all be used to achieve impairing of gene functions at predefined genomic loci (SDN-1) and intended targeted mutations (SDN-2) and precise insertion of a transgene (SDN-3), ODM is practically applied only to generate targeted gene modifications which resemble those of the SDN-2 Type." [line 241, after first bullet, insert further text:] "Prime editing is at a proof-of-concept stage which has to be further developed and assessed, also in regard to unintended and undesired side effects." [Line 247: add further text at the end, after Hua et al., 2019]: ", Agapito-Tenfen et al., 2018; Kawall, 2019; Eckersdorfer et al., 2019; Modrzejewski et al., 2019). In this context, it has to be emphasized that SDN-1 and SDN-2 are the most frequently applied	The GMO Panel considers that for the comments related to lines 211, 220, 227, 235-238, 241, and 247 an explanation of the rational for the proposed change is lacking. Therefore, the proposed changes have not been integrated in the text of the opinion.	123

		genome editing applications, whereby CRISPR/Cas technology is predominant (Modrzejewski et al., 2019; Eckerstorfer et al 2019; Kawall et al., 2020). Depending on the specific SDN-1 or SDN-2 application, more extensive overall changes are possible and involve, for example, multiplexing which targets several genes at once, or repeated applications of SDN-1 or SDN-2 (Zetsche et al., 2017; Raitskin and Patron, 2016). Genome editing opens up new possibilities by making the whole genome accessible for changes (Kawall, 2019; COGEM, 2019, Duensing et al. 2018; Ding et al., 2019). In consequence, the application of SDN-1 and SDN-2 will result in new combinations of genetic information, due to the specific pattern of genetic change. The intended genetic alterations of SDN-1 interventions often show specific patterns because the applied nucleases will typically cut all (or at least many) copies of the target gene(s) throughout the genome. For example, TALENs was used in sugar cane to change 107 out of 109 gene copies of one gene to improve its quality as agro-fuel (Kannan et al., 2018). Furthermore, so-called multiplexing might be applied, which means that not just one, but several genes will be affected (Shen et al., 2017). These examples illustrate the high potential of SDN-1 processes to penetrate the genome and cause profound alterations in the biological characteristics of plants without introducing any additional DNA sequences. The resulting patterns of genetic change as well as biological characteristics and associated risks can be substantially different compared to those derived from previously used methods of breeding (see below). These findings may pose challenges for the comparative approach (EFSA, 2010; EFSA 2011, EU Commission, 2013) which can also go beyond those resulting from transgenesis and SDN-3 (see below).” [Additional text for next chapter: 3.1.3 Methods for delivering or expressing SDN in plants:] [line 263, after bullet add further text – first part, second part see below, next chapter]: "As can be concluded from existing cases of non-regulation in the US and summarized by Testbiotech (2020) and Kawall et al. (2020), in most cases, the 'old', non-targeted techniques of genetic engineering (such as transformation by agrobacterium and biolistic methods) are used in a first step to insert the SDN components into the cells. Further, even if transient methods are used, DNA-free delivery is still a rare exception. In this context, the balance between the intended on-target efficiency and unintended off-target effects is important: in transient applications the components of the SDNs might be degraded too quickly, before the desired genetic changes are achieved. To avoid such failures of the process, higher dosages of the biological mutagens might be applied to the cells, which in response can also give rise to a higher number of unintended effects (Pattanayak et al., 2013; Hsu et al., 2013). Therefore, permanent expression of the SDN machinery in the cells can be a technological advantage, although it requires the establishment of transgenic plants as a first step. A similar situation occurs with ODM which can be inserted via biolistic methods or polyethylene glycol (PEG) (Dong et al., 2006; Pierce et al., 2003; Okuzaki & Toriyama, 2004). It is known from work in animal cells that the insertion of high dosages of oligonucleotides can cause unintended damage on the level of the DNA (Bonner et al., 2012; Olsen et al., 2009)."	The GMO Panel agrees with this comment. Classical breeding techniques (e.g. marker assisted selection) allow also the association of multiple traits (mutations) in a given variety. The GMO panel agrees that this simultaneous association can be achieved potentially more rapidly using SDN-based techniques. This aspect has been taken into consideration in this opinion. The GMO Panel acknowledges that the choice of comparator could be more difficult in case of complex traits expressed by the edited plant. However, it should be noted that complex traits which affect for example the plant metabolism could be also achievable by conventional breeding and traditional transgenesis; hence, this is neither a novel scenario nor a new hazard which is limited only to genome edited plants. The GMO Panel would also like to remind that the "case-by-case" approach as described in the opinion on SDN3 is also applicable to genome edited plants. The GMO Panel considers that for the comments related to line 263 an explanation of the rational for the proposed change is lacking. Therefore, the proposed changes	

			have not been integrated in the text of the opinion.	
International Seed Federation	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	There seem to be two contradictory statements about ODM's; "ODM technology has been only used to generate GM plants." and "ODM is practically applied only to generate targeted gene modification which resembles those of the SDN-2 type". We believe that the latter statement is the correct one.	The GMO Panel reminds that under the current EU regulatory frame, the application of ODM technique gives rise to plants which are considered GMOs.	124
Società Italiana di Genetica Agraria - Italian Society of Agricultural Genetics (SIGA)	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	Line 214 "transcription activator-like effectors (TALENs)" Add "nucleases": "transcription activator-like effectors nucleases (TALENs)".	The text of the opinion has been amended by adding the term "nucleases" after "transcription activator-like effectors".	125
GenØk-centre for biosafety	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	For details regarding this section: please read our attached table with our comments. **Copied from the submitted pdf file:** EFSA fails in performing a uncertainty analysis for the limited scientific information available EFSA recognizes the limited amount of scientific evidence towards certain types of new mutagenesis techniques. However, instead of addressing such lack of knowledge by performing an uncertainty analysis, EFSA is satisfied with current scientific information and further provides its opinion on their hazard identification. According to EFSA's own guidance on uncertainty analysis, EFSA should "identify limitations in scientific knowledge and evaluate their implications for scientific conclusions"3. Although the current document is not a scientific assessment in itself, its conclusion should provide reliable information for EC decision-making, and therefore, any lack of knowledge should be addressed as uncertainty. References are also made to other reviews of the technologies, such as CRISPR which were not included in EFSA's previous opinion. However, as a technical opinion document, EFSA should summarize the relevant information from the scientific literature to inform EC and provide robust evidence for its conclusions within this document. For instance, it is suggested that EFSA provides a box or a table containing the types of CRISPR systems and available Cas nucleases, their functions and expected outcomes following the below criteria: 1) How are these nucleases or oligonucleotide produced? Do they apply recombinant nucleic acids? 2) What are the delivery systems of these mutagenic agents (link to the next section)? 3) What are the biochemical pathways triggered after the incorporation of these mutagenic agents inside the host cell? 4) Is it capable of continued propagation? Is it a heritable material? ODM do not only create SDN-2 type modification Oligonucleotides techniques are characterized by the sequence-specific interaction of nucleic acids, also called hybridization, in vivo. Therefore, parameters, such as the number of nucleotides and range of mutations, are useful guidelines but not exhaustive. There is also the possibility to introduce insertions and deletions.4 In summary, ODM techniques can create all SDN-1, SDN-3 and SDN-3 outcomes but at the same time it is a technique complete different from those as it does not imply the use of any nuclease. EFSA should describe in detail the differences between these techniques.	Comprehensive review of SDN-1, SDN-2 and ODM technologies was not requested by the mandate. Nevertheless, the EFSA GMO panel in its opinion on SDN-1, SDN-2 and ODM technologies provided a brief overview of the technology development since the issue of SDN-3 opinion. This overview is supported by relevant representative original research studies. The limitations and knowledge gaps, that can potentially lead to uncertainty, were identified and are briefly described in the text of the opinion.	126

		How to differentiate SDN-2 and SDN-3? Whereas the main difference for SDN-1 techniques relies on the lack of foreign donor DNA template, it is not clear what are the boundaries for the categorization of SDN-2 and SDN-3.		
Envirnonmental association Za Zemiata	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	[Line 211: Exchange heading]: "Technology used in SDN-1, SDN-2, and ODM applications, its potentials and its restrictions" [line 220: insert subheading:] "3.1.2.1: Overview of current applications" [line 227, after brackets, replace: instead of "...but also to enhance important agronomic traits in crops like.. "]: "… but also to alter relevant traits of important crops like .." [line 235: insert subheading:] "3.1.2.2: Overview of potentials and restrictions" [Line 235-238 insert changed text instead of 'random':] "It should be noted that while ZFNs, TALENs, meganucleases, and CRISPR-Cas system can all be used to achieve impairing of gene functions at predefined genomic loci (SDN-1) and intended targeted mutations (SDN-2) and precise insertion of a transgene (SDN-3), ODM is practically applied only to generate targeted gene modifications which resemble those of the SDN-2 Type." [line 241, after first bullet, insert further text:] "Prime editing is at a proof-of-concept stage which has to be further developed and assessed, also in regard to unintended and undesired side effects." [Line 247: add further text at the end, after Hua et al., 2019]: ", Agapito-Tenfen et al., 2018; Kawall, 2019; Eckersdorfer et al., 2019; Modrzejewski et al., 2019). In this context, it has to be emphasized that SDN-1 and SDN-2 are the most frequently applied genome editing applications, whereby CRISPR/Cas technology is predominant (Modrzejewski et al., 2019; Eckerstorfer et al 2019). Depending on the specific SDN-1 or SDN-2 application, more extensive overall changes are possible and involve, for example, multiplexing which targets several genes at once, or repeated applications of SDN-1 or SDN-2 (Zetsche et al., 2017; Raitskin and Patron, 2016). Genome editing opens up new possibilities by making the whole genome accessible for changes (Kawall, 2019; COGEM, 2019, Duensing et al. 2018; Ding et al., 2019). In consequence, the	The GMO Panel considers that for the comments related to lines 211, 220, 227, 235-238, 241, and 247 an explanation of the rational for the proposed change is lacking. Therefore, the proposed changes have not been integrated in the text of the opinion. The GMO Panel agrees with this comment.	127

		application of SDN-1 and SDN-2 will result in new combinations of genetic information, due to the specific pattern of genetic change. The intended genetic alterations of SDN-1 interventions often show specific patterns because the applied nucleases will typically cut all (or at least many) copies of the target gene(s) throughout the genome. For example, TALENs was used in sugar cane to change 107 out of 109 gene copies of one gene to improve its quality as agro-fuel (Kannan et al., 2018). Furthermore, so-called multiplexing might be applied, which means that not just one, but several genes will be affected (Shen et al., 2017). These examples illustrate the high potential of SDN-1 processes to penetrate the genome and cause profound alterations in the biological characteristics of plants without introducing any additional DNA sequences. The resulting patterns of genetic change as well as biological characteristics and associated risks can be substantially different compared to those derived from previously used methods of breeding (see below). These findings may pose challenges for the comparative approach (EFSA, 2010; EFSA 2011, EU Commission, 2013) which can also go beyond those resulting from transgenesis and SDN-3 (see below)."	Classical breeding techniques (e.g. marker assisted selection) allow also the association of multiple traits (mutations) in a given variety. The GMO panel agrees that this simultaneous association can be achieved potentially more rapidly using SDN-based techniques. This aspect has been taken into consideration in this opinion. The GMO Panel acknowledges that the choice of comparator could be more difficult in case of complex traits expressed by the edited plant. However, it should be noted that complex traits which affect for example the plant metabolism could be also achievable by conventional breeding and traditional transgenesis; hence, this is neither a novel scenario nor a new hazard which is limited only to genome edited plants. The GMO Panel would also like to remind that the "case-by-case" approach as described in the opinion on SDN3 is also applicable to genome edited plants.	
BUND e.V. / Friends of the Earth Germany	3.1.2 Technology used in SDN 1, SDN 2, and ODM applications	Line 227 ADD between "also .. to" the word "trying" (new sentence: ..but also trying to enhance..) Line 236 ADD between "and ..precise" the words "a more or less (Eckerstorfer et al. 2019)" (new sentence: .. targeted mutations and a more or less (Eckerstorfer et al. 2019) precise insertion of ..) Line 239 DELETE "can be used" , ADD "are meant" (new sentence: .. are meant to introduce ..)	The GMO Panel provided examples of CRISPR-Cas systems used to enhance important agronomic traits existing in the literature. The sentence has been revised to improve clarity. The GMO Panel considers that for the comments related to lines 236 and 239 an explanation of the rational for the proposed change is lacking. Therefore, the proposed	128

			changes have not been integrated in the text of the opinion.	
ENSSER	3.1.3 Methods for delivering or expressing SDN in plants	Please make new paragraphs for the various delivery methods. L250: Whilst the term and fact of "DNA-free" is an important aspect, so is the term and fact of "nucleic-acid-free", which is also of relevance in risk assessment, and would further be in line with the Cartagena Protocol. ODM can for obvious reasons not claim to be either. L251: The term "SDN module" is not clear. Does it refer to the actual molecule/complex that is shuttled into the cell via "DNA-free" delivery? L252: The statement that SDNs can be removed by segregation and thus "leaving only the intended sequence mutation in the genome of the final product" is incorrect. This ignores the fact of near-target and off-target mutations, including large deletions and genomic rearrangements (e.g. Kosicki et al., 2018), process induced genome wide mutations and epigentic alterations. It also ignores the insertion of DNA sequences obtained during the delivery or treatment process, including sequences of the carrier plasmid (see also comments and examples for Line 362) Please add the words "theoretically and ideally" for the sentence to read: "….segregation, theoretically and ideally leaving only the intended sequence mutation in the genome of the final product" L255: Is 'transient expression' indeed a "valid alternative" ? It is not a simple process and has its own categories of risk. Thus the methodology should be explained here in much more detail, including its, behaviour, predictability and risk aspects such as the insertion of sequences of the expression construct or vector into the plant genome (Eckerstorfer et al. 2019, or for description see Chen et al. 2019) L263: This section on "DNA free" delivery is missing a few major points related to risk. The methodology description should offer more detail as to how ribonucleoprotein complexes are "directly delivered into the plant cell" as this is relevant for risk and unintended effects. Commonly this requires exposing protoplasts to solutions containing the agent. Whilst protoplast and tissue culture based processes are already prone to induce mutations (see also line 326), there is additionally the possibility of the uptake and integration of any DNA sequences present in the medium, were they to be present from the ribonucleoprotein production (insufficient purification). See also Bertheau (2019). See also references and examples in comments on Line 362. There is also the question as to which extent "DNA"-free is suffient as compared to 'nucleic acid' free (see Line 250). L263: please make new paragraph for ODM L270: Table 1. This table is unfortunately somewhat superficial and oversimplified, that it has little value other than giving rise to wrong assertions or associations. The term "Exogenous DNA" = "DNA originating outside the plant which can be introduced naturally	Please note that the GMO Panel was not mandated to provide a comprehensive literature review on the technology available for SDN-1, SDN-2, and ODM. The section is meant to provide some background information which can be useful in order to understand the following sections. Regarding comment to line 250, the GMO Panel takes note of the comment. Regarding comment to line 251, the term "SDN module" is defined as "the molecular components necessary to achieve the genetic mutation". Regarding comment to line 252, the phrase "*leaving only the intended sequence mutation in the genome of the final product*" has been removed from the text. Regarding comment to line 255, the GMO Panel considers the comment out of the scope of the mandate. The GMO Panel was not mandated to provide a detailed description of all the methodologies deployed to produce a genome edited plant. Regarding comment to line 263, by definition the GMO panel considers that plants obtained by SDN-1, SDN-2, and ODM technologies are not transgenic. Establishment of the techniques and data needed to demonstrate	129

		or by technological intervention" is described differently here than in the glossary. Furthermore, it is commonly understood that the exogenous DNA may have a sequence equivalent in the recipient cell, but it is being added/intorduced from the outside via technological intervention, this also referring to gene shuttles such as Agrobacterium. It would be good to clarify this in the glossary. Term "delivery methods" as heading does not match the entries. Delivery of what? What is meant by "SDN module"? The DNA construct, ie the expression construct? (see also comment and question Line 251, where it was supposedly defined) And why only looking at the SDN module as such? What about components of it? Or components of backbones? This gives a false sense of certainty. See lines 281/2, where it is explicitly said, but faiils to do in the table. Please amend The legend would need to be written differently also to explained that this is an 'intention' table, and not a depiction of reality. Description L 282 different to table.	that the produced plant is not transgenic is not in the remit of the given mandate. The GMO Panel considers that for the comments related to lines 263 (i.e. the insertion of a new paragraph for ODM method), an explanation of the rational for the proposed change is lacking. Therefore, the proposed changes have not been integrated in the text of the opinion. Regarding the comment related to Table 1, please note that the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader. Please note that "SDN module" is define in 3.1.3 as the molecular components necessary to achieve the genetic mutation. Depending on the gene modification strategy it can be constituted of proteins and/or RNA and/or DNA and/or chimeraplast.	
EuropaBio	3.1.3 Methods for delivering or expressing SDN in plants	Line 249. A definition of "site directed mutagenesis" would be useful here. Line 270. Table-1: the purpose of this table is not clear to EuropaBio. Throughout the document the terms "exogenous" and "foreign" are used interchangeably (e.g. lines 315-317, which indicates that SDN1, SDN2 and ODM do not contain "exogenous" DNA vs lines 207-210 where SDN1, SDN2 and ODM are described not containing "foreign" DNA). The last column makes reference to "exogenous DNA", but it is not clear what this is. Our understanding is that this could be clarified by using the term "foreign DNA". Since EFSA indicates in several occasions that SDN1-SDN2-ODM plants are free of transgenic sequences, then it is important to clearly identify in what context the term exogenous is used (Table 1 defines it as any DNA sequence generated from outside the plant, but that does not mean those sequences are necessarily transgenic). A better explanation and more context on this Table should be provided or it should be deleted all together.	Regarding the comment related to Table 1, please note that the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	130
Association Française de	3.1.3 Methods for delivering	AFBV edits and comments on Table 1: • In the 3rd column, remove parenthesis "(only applicable for sexually reproducing plants)" and	Regarding the comment related to Table 1, please note that the table has been removed because i) it	131

Biotechnologies Végétales	or expressing SDN in plants	replace with "or removed by molecular excision". We suggest placing** after "excision" and adding a footnote** as follows: An example is described for apple cultivars: Pompili et al. 2020. • In the 4th column, replace "Yes" with "No" at the level of ODM: no Exogenous DNA is used in the ODM technology (large synthesized oligonucleotides of more than 20 to 100 nucleotides in length are used and no DNA is cut). EUROPEAN COMMISSION (2017) at p. 63.	did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	
Federal Office of Consumer Protection and Food Safety (BVL), Competent Authority according to Directive 2001/18/EC	3.1.3 Methods for delivering or expressing SDN in plants	Line 270, Table 1: In the third column the phrasing " No, if crossed out" should be replaced by "No, if segregated out". Segregant populations can be obtained not only by crossing, but also by selfing, if the insertion of the SDN module occurred heterozygously (which is usually the case).	Regarding the comment related to Table 1, please note that the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	132
Julius Kühn-Institut	3.1.3 Methods for delivering or expressing SDN in plants	L270: For clarity consider to reformat the table: i) in the header of the last column consider to replace "deployed" by "introduced into the cell" as the "process" is not clearly defined here and may be interpreted as also comprising any step prior to delivery. ii)) In the second row of the column the text in brackets may be deleted or transferred to the table legend (as sexual reproduction is a logical limitation, it can be removed from the table for more clarity) iii) In row 4 and column 4 of the table: consider to omit "(if synthetized RNA is used)" because actually the table does not refer to any NA contaminations and purification steps preparing the components for the delivery. You may clarify it in an explanatory sentence in the text. You should also stay consistent with the description of DNA-free delivery in line 256. iv) Switch the second-last and the last column for a more logic order.	Regarding the comment related to Table 1, please note that the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	133
Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG)	3.1.3 Methods for delivering or expressing SDN in plants	Table 1: 3rd column: maybe delete the sentence in the brackets	Regarding the comment related to Table 1, please note that the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	134
Scientific Committee for GM food and Feed, Advisory Body, Czech Republic	3.1.3 Methods for delivering or expressing SDN in plants	Document states that SDN module can be removed by segregation, but "This step is not possible in case of non-sexually propagated crops (for example, for vegetatively propagated crops)." This wording is not accurate because some vegetatively propagated crops (e.g. potatoes) are sexually crossed in the breeding process, so even in these crops the module can be removed by segregation. The wording used should be more specific.	The text has been revised to indicate that the step "may not likely be performed in case of commonly asexually (vegetatively) propagated crops".	135

		Table 1: For stable integration (in the first line, second column), it would be more appropriate to state that "Yes/No (the SDN module can be removed by segregation in sexually reproducing crops). For DNA-free delivery, in the last column it should be stated simply "No" or alternatively "No (the executive protein itself and/or RNA is delivered)".	Regarding the comment related to Table 1, please note that the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	
Plantum - Netherlands seed association	3.1.3 Methods for delivering or expressing SDN in plants	Line 253. Very few vegetatively propagated crops do not set seed (e.g. triploid banana). Most can be (and commonly are in breeding) sexually propagated. This means that the step referred to could be applied. It may however not happen often, since a major advantage of mutation breeding is that the genetic background of the variety remains intact. We therefore suggest to rephrase the sentence as follows: "This step may not likely be performed in case of commonly vegetatively propagated crops."	Regarding comment to line 253, the GMO Panel thanks for the comment. The text has been amended by indicating "This step may not likely be performed in case of commonly asexualy (vegetatively) propagated crops" in order to improve clarity.	136
French agency for Food, Environmental and Occupational Health & Safety (Anses)	3.1.3 Methods for delivering or expressing SDN in plants	Page 7, lines 251-254: The sentences "In case of stable integration and for sexually propagated crops, the SDN module can be removed by segregation […]. This step is not possible in case of non-sexually propagated crops (for example, for vegetatively propagated crops)." look contradictory with those of lines 160-163, which suggest that it is always possible. Again, the removal of the SDN genes by segregation must be demonstrated or, when this removal is not feasible, the associated potential hazards need to be studied (see comments on lines 160-163 and 169-170). Page 7, line 255: "… transient expression is a valid alternative method": same comment as on lines 160-163, the transient expression of SDN must be demonstrated. Page 8 lines 270-271: Table 1, line "DNA-free delivery", column "Exogenous DNA* deployed at any stage during the process": proposal to replace "No (if synthetized RNA is used)" by "No (if synthetized RNA, the protein itself (for TALENs, ZFNs, and meganucleases), or the ribonucleoprotein complex (for CRISPR-Cas system) is used", for consistency with lines 257-258.	Regarding comment to lines 251-254, the GMO panel considers that plants obtained by SDN- and ODM-based technologies are not to be considered transgenic. Moreover, the establishment of the techniques and data needed to demonstrate that the produced plant does not contain any transgene is not in the remit of the given mandate. Please note that the opinion includes already a statement clarifying that the applicant should demonstrate the absence of any integrated exogenous DNA, should the final product be not intended to retain it. Regarding comment to line 255, please, consider the response provided above for comment to line 160-163. Regarding the comment related to Table 1, please note that the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it	137

			was considered not clear enough for the reader.	
Corteva Agriscience	3.1.3 Methods for delivering or expressing SDN in plants	Lines 253-254. Suggest changing "for example, for vegetatively propagated crops" to "i.e., for vegetatively propagated crops". There are only two types of propagation: sexual and asexual (vegetative); vegetative propagation includes various methods. Lines 254-256: addition proposed: "In the case when the presence …, transient expression or DNA-free delivery are valid alternative methods…" Lines 266-268. The statement appears (and needs to) refer to delivery methods for all genome editing techniques, however referenced Metje-Sprink et al, 2019 is about DNA-free methods only. Suggest adding to the list Agrobacterium-mediated transformation [e.g., 1;2] and viral-based delivery [e.g., 3;4] also used in DNA-based delivery. 1. Liang Z, Zhang K, Chen K, Gao C. (2014) Targeted mutagenesis in Zea mays using TALENs and the CRISPR/Cas system. Journal of Genetics and Genomics 41:63-8. 2. Anand A. and Jones T.J. (2018) Advancing Agrobacterium-based crop transformation and genome modification technology for agricultural biotechnology. In: Current topics in microbiology and immunology. 418: 489-507. doi 10.1007/82_2018_97. 3. Ali Z, Abul-faraj A, Li L, et al. (2015) Efficient Virus-Mediated Genome Editing in Plants Using the CRISPR/Cas9 System. Molecular Plant 8: 1288-1291. 4. Butler N.M., Baltes, N.J., Voytas D.F., and Douches, d.S. (2016) Geminivirus-mediated genome editing in potato (Solanum tberosum L.) using sequence-specoific nucleases. Frontiers in Plant Science. Doi: 10.3389/fpls.2016. 01045. Line 270 table: The title of EFSA's Table 1 says it's a summary of delivery methods for the SDN and ODN available in plants– so it is not clear how the "exogenous DNA" column is to be understood. Internal discussions showed that this column was interpreted differently by different people and therefore we would ask that it is deleted from this Table. Furthermore, the field is still evolving and such a table might give the impression that techniques can be easily separated while this will become more and more difficult as the options further expand. In order to avoid a need for continuous updates we would suggest to put the Table in a broader context of continuous innovation or to delete it in its entirety.	Regarding comment to lines 253-254, the GMO Panel thanks for the comment. The text has been amended accordingly. Regarding comment to lines 254-256, the GMO Panel thanks for the comment. The text has been amended accordingly. Regarding comment to lines 266-268, a new reference on genome editing techniques has been added to the text. Regarding the comment related to Table 1, please note that the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	138
European Plant Science Organisation, EPSO	3.1.3 Methods for delivering or expressing SDN in plants	Line 270 to 272 (Table 1.): It is unclear what the header in column four refers to. Consider using "introduced into the cell" instead of "deployed", as the term "process" may refer to what happens on the lab-bench before delivery. As stated on line 256 to 257 DNA-free delivery systems may consist of proteins only in case of, e.g. TALENs. Consider removing "(if synthetized RNA is used)". In addition, the mention of "RNA" in a column referring to "DNA" is confusing.	Regarding the comment related to Table 1, please note that the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	139
Haut Conseil des biotechnologies	3.1.3 Methods for delivering	l. 253-254. "This step is not possible in case of non-sexually propagated crops (for example, for vegetatively propagated crops)."	Regarding comment to lines 253-254, the GMO Panel thanks for the	140

(High Council for Biotechnology)	or expressing SDN in plants	Can it be unequivocally said that backcrossing is not possible for non-sexually propagated crops? This can be misleading. It seems to us that, while some varieties may show complete sterility, there is usually no strict biological or technical impossibility to crossing most vegetatively propagated crops, but that backcrossing is avoided for the reason that it would further modify the genetic makeup of valuable, highly characterized, heterozygote genotypes. l. 254-255. "In this case when the presence of the SDN gene cassette in the final product is not desirable, transient expression is a valid alternative method to express the SDN module ". We suggest replacing "is a valid" by "could be a valid". Evidence for the absence of the SDN gene cassette in the plant genome will still have to be brought in the risk assessment process (see 3.2.2.2.2). This section should also make clear that transient expression is also possible for sexually propagated crops.	comment. The text has been revised to reflect the fact that backcrossing is likely not performed with asexually propagated crops. Regarding comment to lines 254-255, the GMO Panel thanks for the suggestion. The text has been revised accordingly. The GMO Panel thanks for the comment. The text has been revised to clarify that transient expression could be a valid approach in all those cases when the presence of the SDN gene cassette in the final product is not desirable.	
Testbiotech	3.1.3 Methods for delivering or expressing SDN in plants	[line 263, after bullet add further text – second part, first part see above, last chapter]: „Whatever the case, starting from the process, the first step of introducing the biological mutagens into the cell has to be considered in risk assessment, since it adds unintended effects, which are not directly caused by the final effectors (the nuclease or the nucleotide) and their intended usages. In this context, it should be taken into account that the transformation via Agrobacterium and biolistic methods can result in complex genetic insertions containing multiple copies of the transgene and/or rearrangements of both the DNA intended to be inserted and the host plant DNA; this can also result in other unintended effects such as epigenetic alterations in the vicinity of the integration site (Forsbach et al., 2003; Jupe et al., 2019; Kim et al., 2003; Latham et al., 2006; Makarevitch et al., 2003; Rang et al., 2005; Windels et al., 2003). Therefore, SDN-1 and SDN-2 plants derived from such processes need to be carefully assessed, including risks associated with the use of the respective transformation method. This also has to be investigated in plants if the transgene was subsequently removed by segregation, since there might be further unintended changes caused by the transformation process and not only by the transgene itself. This is in line with current EFSA practice: transgenic plants which have undergone the process of segregation and therefore no longer inherit the transgenes are not accepted as comparators for risk assessment of transgenic plants." [line 268, after bullet] "Therefore, also in case of ODM, the first step of introducing the molecules into the cell, has to be considered in risk assessment, since it might go along with unintended effects, not directly caused by the final effector." [After Line 273, insert further text:]	The GMO Panel considers that for the comments related to lines 263 an explanation of the rational for the proposed change is lacking. Therefore, the proposed changes have not been integrated in the text of the opinion. The GMO panel considers that plants obtained by SDN- and ODM-based technologies are not to be considered transgenic. Moreover, the establishment of the techniques and data needed to demonstrate that the produced plant does not contain any transgene is not in the remit of the given mandate. Please note that the opinion includes already a statement clarifying that the applicant should demonstrate the absence of any integrated exogenous DNA, should the final product be not intended to retain it.	141

		"3.1.4 Summary of technological aspects with relevance for risk assessment of SDN-1, SDN-2 and ODM In general, the application of SDN-1, SDN-2, and ODM methods results in an intended modification or an impairment of gene functions at predefined genomic loci without the insertion of foreign DNA. From the description of the technology, there are several findings that have to be taken into account within the ToR of the mandate: • The applied technical processes can be divided into several steps: (1) The delivery of the SDN components or ODM molecules into the plant cells; (2) the processes caused by the effectors (biological mutagens), followed by (3) further breeding which is, e.g. used to segregate transgenes or other unintended genetic alterations. The first two steps involve technical steps that have to be considered as potential causes of hazards (risks) while the last step is meant to mitigate or eliminate some of the risks. Therefore, all steps of the process have to be considered in the risk assessment and assessed on the basis of sufficiently comprehensive data. • SDN-1 and SDN-2 applications typically result in new combinations of genetic information due to the specific patterns of genetic change. Existing examples show SDN-1 and SDN-2 applications have a high potential to penetrate the genome and cause profound alterations in the biological characteristics of plants without introducing any additional DNA sequences. The resulting unintended and intended biological characteristics may pose substantial challenges for the comparative approach as currently applied (see, for example, EFSA, 2010). These findings have to be added to the risk assessment of specific unintended effects (on-target and off target), which are caused by the specific processes of the biological mutagens and are discussed below."	By definition the GMO panel considers that plants obtained by SDN1/SDN2/ODM technologies are not transgenic. Establishment of the techniques and data needed to demonstrate that the produced plant is not transgenic is not in the remit of the given mandate The GMO Panel considers that for the comments related to lines 273 an explanation of the rational for the proposed change is lacking. Therefore, the proposed changes have not been integrated in the text of the opinion.	
Umweltbundesamt (Environment Agency Austria) on behalf of the Austrian lead Competent Authority, the Federal Ministry of Social Affairs, Health, Care and Consumer Protection.	3.1.3 Methods for delivering or expressing SDN in plants	Line 249ff: The discussion of delivery methods for SDN-components is appreciated. However, the discussion presented in the draft opinion is not ad-dressing a number of important points: * All mentioned delivery methods can induce unintended genetic changes in their frame of the necessary workflow, involving cell or tissue culture, regeneration of plantlings, etc. in addition to off-target changes introduced by the SDN-system (Eckerstorfer et al., 2019a). Assessment and removal of unwanted changes requires selection procedures comparable to the ones used for getting rid of secondary mutations in classical mutagenesis approaches in conventional breeding. * Furthermore it needs to be mentioned that most of the current applications picked up in recent reviews use stable transformation of the genetic con-structs for expression of SDN components, rather few use transient expression, still fewer DNA-free delivery methods (Eckerstorfer et al., 2019a; Modrzejewski et al., 2019). * Finally, the information in Table 1 (Line 270f) needs to be revised: Without experimental confirmation during risk assessment the conclusions presented in column 3 are assumptions rather than facts with a view to individual applications of genome editing. The information presented in the Table disregards that there is a chance that transiently present expression constructs or oligonucleotides may be integrated into the genomic DNA of the modified plants at low, but not negligible frequencies. It further omits that the removal of stably integrated expression constructs as	The GMO panel agrees with this comment. Indeed, the GMO Panel concluded that no additional hazard associated with the use of the SDN-1, SDN-2 and ODM approaches could be identified as compared to both SDN-3 and conventional breeding techniques, including conventional mutagenesis. The GMO Panel was not mandated to produce a literature review on the technology used in SDN- and ODM-based approaches and their application. Regarding the comment related to Table 1, please note that the table has been removed because i) it	142

		well as of other unintended genetic changes introduced during the engineering process cannot be taken for granted, but needs to be verified during risk assessment. The important issue is summarized in column 4: All methods use exogenously made DNA or RNA constructs that might integrated into the genome of the edited plants by one way or the other (the hairsplitting around the wording of the EC document is not considered helpful here). Eckerstorfer, Michael F.; Dolezel, Marion; Heissenberger, Andreas; Miklau, Marianne; Reichenbecher, Wolfram; Steinbrecher, Ricarda A.; Waßmann, Friedrich (2019a): An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs). Frontiers in bioengineering and bio-technology 7, S. 31. DOI: 10.3389/fbioe.2019.00031. Modrzejewski, D.; Hartung, F.; Sprink, T.; Krause, D.; Kohl, C.; Wilhelm, R. (2019): What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a sys-tematic map. In: Environ Evid 8 (1). DOI: 10.1186/s13750-019-0171-5.	did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	
International Seed Federation	3.1.3 Methods for delivering or expressing SDN in plants	The intention of the table requires further clarity especially on the purpose of the last column. It is difficult to understand the message of this column.	Regarding the comment related to Table 1, please note that the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	143
Società Italiana di Genetica Agraria - Italian Society of Agricultural Genetics (SIGA)	3.1.3 Methods for delivering or expressing SDN in plants	Line 253-254 "This step is not possible in case of non-sexually propagated crops (for example, for vegetatively propagated crops)". We consider that even for non-sexually propagated crops, sexual segregation of the SDN module is possible, even though it is often undesirable due to segregation in the sexual progeny and consequent impossibility to recover the original genotype. However, the value of the introduced trait can justify the effort of selecting the desired recombinants in the progeny. We suggest this rephrasing: "In case of non-sexually propagated crops (for example, for vegetatively propagated crops) this step, though possible, is often not practical".	The GMO Panel thanks for the comment. The text has been revised to reflect the fact that backcrossing is likely not performed with asexually propagated crops.	144
• Cornell University's Alliance for Science	3.1.3 Methods for delivering or expressing SDN in plants	The Panel rightly distinguished the delivery methods for SDN and ODM techniques further clarifying the different possibilities regarding the end product's genetic composition. The GMO Panel correctly used these distinctions when evaluating the applicability of "Section 4" and the "Conclusions" of the EFSA opinion on SDN-3 to plants developed using SDN-1, SDN-2, and ODM techniques.	The GMO panel thanks Environmental Resources, Costa Rican Association of Food Technology, Costa Rica for its comment.	145
GenØk-centre for biosafety	3.1.3 Methods for delivering	For details regarding this section: please read our attached table with our comments. Copied from the submitted pdf file:	Regarding the comment related to Table 1, please note that the table has been removed because i) it	146

	or expressing SDN in plants	Table should focus on the characteristics of the techniques not the final product EFSA provides a table summarizing the differences in delivery methods for the different techniques of gene editing. Instead, EFSA should present information related to the following criteria which could be included as columns after the column 'delivery methods': 1) What biological material will be delivered inside the cell? Nucleic acids (DNA or RNA or both), proteins or both? 2) What supporting techniques could be applied for efficient delivery (electroporation, lipofectamine, other mutagenic reagent)? Are they mutagenic reagents or techniques? Do they apply genetic engineering techniques? 3) Link the information to the categories SDN-1, SDN-2 and SDN-3. It is also suggested that the two last columns are deleted as they refer to the final product, which should be tested during RA (column 3) or limited to DNA presence and does not include all nucleic acids (column 4). In Annex I, there is a suggestion for a new Table containing the above-mentioned criteria. Supporting techniques for gene editing EFSA spends 10 lines out of the 20 lines in this section to discuss DNA-free delivery methods. As suggested in our Annex I, DNA-free delivery methods are only one type of delivery and it can only achieve SDN-1. In addition, EFSA does not explore the supporting techniques used in conjunction to these gene editing technologies. Most of these technologies rely on the use of supporting techniques for their success. And depending on techniques used, the organism might be risk assessed differently. Therefore, it is suggested that EFSA describes all delivery methods available this far, which are the supporting techniques used in these, to which degree they have been applied in plants and which ones are more likely to reach market in the near future. A comprehensive list of the possibilities of delivery methods for each SDN type and the supporting techniques is provided in Table 1 in Annex I of this document.	did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader It should be noted that the GMO Panel was not mandated to produce a literature review on the technology used in SDN- and ODM-based approaches, including the frequency of their application.	
Federal Agency for Nature Conservation	3.1.3 Methods for delivering or expressing SDN in plants	Line 252: Upon transformation using Agrobacterium tumefaciens or particle gun whole genes, but also fragments of SDN modules can be integrated and should be removed. However, if removed, this is no sufficient reason to lessen guidance requirements as the developed plants can still contain unintended effects caused by the transformation process. This is the reason why EFSA does not accept null segregants as only comparators in risk assessment. Please incorporate this aspect in the draft. Line 249-273: This paragraph along with Table 1 gives the impression that the DNA-free delivery is equally used as the other delivery methods. According to the scientific literature considered by Eckerstorfer et al. (2019) SDN techniques are not used in isolation, but combined with various biotechnological methods in the breeding processes involving in many cases classical genetic engineering at some point to establish intermediate or final products containing transgenic insertions. Please consider this aspect and adapt the text accordingly.	The GMO Panel would like to clarify that unintended effects expected in transgenic plants are necessarily linked to the transgene itself. In risk assessing a transgenic plant, the GMO Panel gives an opinion on the event(s). This event is then supposed to be introgressed in many varieties eliminating possible unintended effects caused by the transformation process and which are not genetically linked to the transgene. The GMO panel considers that plants obtained by SDN- and ODM-based technologies are not to be considered transgenic. Moreover, the establishment of the techniques and data needed to	147

			demonstrate that the produced plant does not contain any transgene is not in the remit of the given mandate. Please note that the opinion includes already a statement clarifying that the applicant should demonstrate the absence of any integrated exogenous DNA, should the final product be not intended to retain it. The GMO Panel does not consider that DNA-free delivery is used with the same frequency as the other delivery methods. The paragraph is meant just to provide an overview on the available methods used to deliver the SDN module. It should be noted that the GMO Panel was not mandated to produce a literature review on the technology used in SDN- and ODM-based approaches, including the frequency of their application. Regarding the comment related to Table 1, please note that the table has been removed because i) it did not add additional information which was not already included in the text of the section and ii) it was considered not clear enough for the reader.	
Envirnonmental association Za Zemiata	3.1.3 Methods for delivering or expressing SDN in plants	[line 263, after bullet add further text]: "As can be concluded from existing cases of non-regulation in the US and summarized by Testbiotech (2020), in most cases, the 'old', non-targeted techniques of genetic engineering (such as transformation by agrobacterium and biolistic methods) are used in a first step to insert the SDN components into the cells. Further, even if transient methods are used, DNA-free delivery is still a rare exception. In this context, the balance between the intended on-target efficiency and unintended off-target effects is important: in transient applications the components of the SDNs might be degraded too quickly, before the desired genetic changes are achieved. To avoid such failures of the process, higher dosages of the biological mutagens might be applied to the cells, which in response can also give rise to a higher number of unintended effects (Pattanayak et al., 2013; Hsu et al., 2013).	Regarding comment to line 263, The GMO Panel takes note of 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	148

		Therefore, permanent expression of the SDN machinery in the cells can be a technological advantage, although it requires the establishment of transgenic plants as a first step. A similar situation occurs with ODM which can be inserted via biolistic methods or polyethylene glycol (PEG) (Dong et al., 2006; Pierce et al., 2003; Okuzaki & Toriyama, 2004). It is known from work in animal cells that the insertion of high dosages of oligonucleotides can cause unintended damage on the level of the DNA (Bonner et al., 2012; Olsen et al., 2009). Whatever the case, starting from the process, the first step of introducing the biological mutagens into the cell has to be considered in risk assessment, since it adds unintended effects, which are not directly caused by the final effectors (the nuclease or the nucleotide) and their intended usages. In this context, it should be taken into account that the transformation via Agrobacterium and biolistic methods can result in complex genetic insertions containing multiple copies of the transgene and/or rearrangements of both the DNA intended to be inserted and the host plant DNA; this can also result in other unintended effects such as epigenetic alterations in the vicinity of the integration site (Forsbach et al., 2003; Jupe et al., 2019; Kim et al., 2003; Latham et al., 2006; Makarevitch et al., 2003; Rang et al., 2005; Windels et al., 2003). Therefore, SDN-1 and SDN-2 plants derived from such processes need to be carefully assessed, including risks associated with the use of the respective transformation method. This also has to be investigated in plants if the transgene was subsequently removed by segregation, since there might be further unintended changes caused by the transformation process and not only by the transgene itself. This is in line with current EFSA practice: plants which have undergone the process of segregation and therefore no longer inherit the transgenes are not accepted as comparators for risk assessment of transgenic plants." [line 268, after bullet] "Therefore, also in case of ODM, the first step of introducing the molecules into the cell, has to be considered in risk assessment, since it might go along with unintended effects, not directly caused by the final effector." [After Line 273, insert further text:]	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. In order to clarify its positions, the GMO Panel has revised the text of the opinion accordingly and included some additional relevant references. The GMO panel considers that plants obtained by SDN- and ODM-based technologies are not to be considered transgenic. Moreover, the establishment of the techniques and data needed to demonstrate that the produced plant does not contain any transgene is not in the remit of the given mandate. Nevertheless, the opinion includes already a statement clarifying that the applicant should demonstrate the absence of any integrated exogenous DNA, should the final product be not intended to retain it. Moreover, The GMO Panel would also like to remind that the "case-by-case" approach as described in the opinion on SDN3 is also applicable to genome edited plants. The GMO Panel would like to clarify that unintended effects expected in transgenic plants are necessarily linked to the transgene itself. In risk assessing a transgenic plant, the GMO Panel gives an opinion on the event(s). This event is then supposed to be introgressed in many varieties eliminating possible unintended	

			effects caused by the transformation process and which are not genetically linked to the transgene. The reason why the GMO panel does not accept null segregants as comparators for risk assessment of transgenic plants is because these plants have experienced a limited number of backcrosses that would not guarantee segregation between the transgene and a potential yet genetically unlinked unintended effect.	
BUND e.V. / Friends of the Earth Germany	3.1.3 Methods for delivering or expressing SDN in plants	Line 253 ADD in the end of the sentence : ", although removal of SDN genes or remnants of the transformation process by segregation may not be achieved if there are multiple integration sites of foreign DNA sequences (Michno et al. 2020)." Line 263 ADD additional sentence between "..and soybean (Kim et al., 2017)." and "In case of ODM..": "These techniques, however, cannot reliably prevent the integration of foreign DNA (Andersson et al. 2018)." Line 269 ADD additional sentence: "Remnants of vector sequences can be integrated at various genomic sites (Braatz et al. 2017)."	The GMO Panel considers that for the comments related to lines 253, 263, and 269 an explanation of the rational for the proposed change is lacking. Therefore, the proposed changes have not been integrated in the text of the opinion.	149
ENSSER	3.2.1 Introduction	Overarching point: SDN1 & 2 give rise to a broad spectrum of scenarios that would require consideration for hazard assessment and risk assessment, including scenarios of complex interventions (Zsogon et al. 2018; Sanchez-Leon et al. 2018; Kannan et al. 2018). These fail to be explored or taken account of in this draft opinion. Oversimplifying, this opinion reduces the assessment to two rather simplistic sub-criteria for consideration: namely to the presence (or lack of presence) of any "exogenous" DNA on one hand, and to the "modification introduced at the target sequence(s)". Risk hypothesis should not be restricted to the presence of foreign nucleic acids, nor to simply the intended trait nor to solely the modifications at the intended target sites (Agapito-Tenfen et al. 2018). Presence of sequences of so-called cis-genes would equally give rise to risk scenarios, depending on what they are, where they are inserted, what they disrupt, and if they have regulatory -including epigenetic- impacts or consequences. Laying out the risk assessment into these two criteria is not providing the clarity and rigour and science required. Indeed, both scenarios are failing to clearly point to off-target effects, unintended on-target effects, or process induced effects. We are missing the assessment and the literature for this.	Section 3.2.1 is meant to provide an introductory part to the assessment of the applicability of the section 4 of the EFSA opinion on SDN-3 to plant developed by SDN-1, SDN-2, and ODM. Clearly, the most obvious difference between SDN-3 and the other techniques is that with the latter ones no exogenous DNA is meant to be integrated in the plant genome. However, this does not exclude the possibility that exogenous DNA could be integrated intentionally (for example, to express the nuclease)	150

		Equally, epigenetic effects are not given the space they should have in such an opinion document. Specifics: L279: please re-state that "conventional bred plants" in this case specifically means mutational breeding techniques that emerged prior 2001. Please list in footnote which ones in specific are being referred to, and the datasets used. Please clarify if those 'emerged' and had a long record and history of safety prior to the adoption of the 2001 Directive. L281: Please, again, clarify (and add to glossary) what is meant by "SDN module" – is it the genetic information at the DNA level coding for a specific SDN molecule, is it the messenger RNA yet requiring translation, or is it the actual SDN molecule itself (ie protein or ribonucleoprotein complex, including the guide RNA)? L281: delete "exogenous" and add "and/or any nucleic acid sequence" and "in its original or altered form" so it reads: "..and/or any DNA sequence or any nucleic acid sequence deployed during the genome editing process is present in whole or in parts in the plant genome." L284: change to genome edited (currently is 'gene edited') L286: please change to: "..and/or any DNA sequence or any nucleic acid sequence deployed during the genome editing process is not present in its original or altered form in the plant genome." L287/8: We expect the opinion and the scenarios to be adjusted and elaborated in line with our overarching point above in this section, as to allow for a robust and reliable risk assessment and to be in line with the precautionary principle and the directive as it stands. Accordingly, for this particular scenario, either delete "In this case, the plant will only be assessed with regards to the modification introduced at the target sequence(s)." or alter it in the following way: "In this case the plant will be fully assessed with regards to any sequence and epigenome modifications resulting from the processes and actions carried out and employed to achieve the alteration at the on-target site. These may also include transformation induced mutations should an SDN DNA construct have been integrated into the genome in previous generations." Please provide the relevant risk research and references, including for on- and off-target effects or make cross-reference as to where to find them in this document.	or unintentionally. The GMO Panel considered important to provide an operational distinction between these two scenarios in order to provide the background for the following sections. It should be noted that both scenarios will be risk assessed. Regarding comment in line 279, a footnote has been inserted in the text to refer to the list of techniques relevant for a comparison as indicated in the opinion on SDN-3. Regarding comment for line 281, the operational definition of "SDN module" used in this opinion has been added to the glossary. The term "exogenous" has been replaced by the proposed sentence. Regarding comment for line 281 and 284, the text has been amended accordingly. Regarding line 286, the text has been amended accordingly. Regarding the comment for line 287/8, the text has been revised to improve clarity.	
Association Française de Biotechnologies Végétales	3.2.1 Introduction	AFBV edit and comment: Line 287: replace "in the plant genome" with "in the genome of the edited plant". The intent of this edit is to make the sentence easier to understand.	The text has been amended accordingly.	151
Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG)	3.2.1 Introduction	line 287 replace plat genome by - in the genome of the gene edited plant	The text has been amended accordingly.	152
GMO Office, National Institute of Public Health and the	3.2.1 Introduction	Line 280-287 EFSA states that there are two scenario's foreseen. The first scenario is when any exogenous DNA deployed during the process is inserted in the genome (intentionally or unintentionally), in that case the plant will be assessed as a transgenic plant. The	Section 3.2.1 is meant to provide an introductory part to the assessment of the applicability of the section 4 of the EFSA opinion	153

Environment (RIVM)		same plants will also be assessed as a gene edited plants with regard to the modification of the target sequence(s). The second scenario is when no exogenous DNA is inserted, in that case the plant will only be assessed as a gene edited plant with regard to the modification of the target sequence(s). We understand that plants that are edited using SDN-1, SDN-2 and ODM are to be assessed as gene edited plants. We also understand that plants intentionally modified with an SDN construct (not segregated) are to be assessed as transgenic plants. What we do not understand is that plants that contain (unintentionally introduced) exogenous DNA fragments deployed during the process are to be assessed as transgenic plants. This seems rather a legal approach than a scientific approach and is confusing for the reader. The opinion could be improved by stating upfront that gene edited plants using SDN-1, -2 and ODM are assumed not to contain any exogenous DNA deployed during the process and that the current EFSA opinion is based on this assumption. Thereafter a section could be included where is mentioned what would be the practical consequence for the risk assessment in case there is exogenous DNA present in the plant. The focus of this opinion should be on the risk assessment of gene edited plants, and not on exogenous DNA that may or may not be present.	on SDN3 to plant developed by SDN1, SDN2, and ODM. Clearly, the most obvious difference between SDN3 and the other techniques is that with the latter ones no exogenous DNA is meant to be integrated in the plant genome. However, this does not exclude the possibility that exogenous DNA could be integrated intentionally (for example, to express the nuclease) or unintentionally. Although the GMO Panel agrees that genome editing techniques aim at modifying the genome without introducing foreign DNA, the panel was not mandated to provide a definition of genome edited plants whose risk assessment falls under the current EU regulation of GMOs (CJEU in Case C-528/16). The GMO Panel considered important to provide just an operational distinction between these two scenarios in order to provide the background for the following sections. Some text refinements have been introduced to improve clarity.	
French agency for Food, Environmental and Occupational Health & Safety (Anses)	3.2.1 Introduction	Page 9, lines 278-279: Again, the sentence "which compares the hazards associated with plants developed using SDN-3 approaches to those derived from transgenic and conventionally bred plants" is not correct, because the hazards are not derived from the plants. Please replace "derived from" by "associated with". Page 9, lines 281-282: "… the full SDN module, part of it, or any exogenous DNA sequence deployed during the genome editing process is present in the plant genome": again, a clarification is needed, to indicate if in this scenario the insertion of all or part of the SDN module is maintained in the final product (see comments on lines 160-163, 169-170 and 251-254). Page 9, lines 287-288: "In this case, the plant will only be assessed with regards to the modification introduced at the target sequence(s).": in case of stable integration followed by removal of the SDN module, the hazards associated to these steps should also be studied.	Regarding comment for line 278-279, the text has been amended accordingly. Regarding comment for line 281-282, the GMO Panel considers the text sufficiently clear. Regarding comment for line 287-288, the sentence has been revised. Regarding this comment, the GMO Panel invites ANSES to refer to the responses given for the comments related to section 3.2.2 and its sub-sections.	154

Corteva Agriscience	3.2.1 Introduction	Lines 285-288. Due to the court decision plants that do not contain the SDN module and any other exogenous DNA sequences are yet to be risk-assessed under the GMO Directive, but in line with the proportionality principle (https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:12012M/TXT&from=en), embedded in key parts of the legislation (include food safety legislation) the assessment should be only targeted to hazards identified based on a credible risk hypothesis.	The GMO Panel thanks Corteva Agriscience and takes note of the comment.	155
Testbiotech	3.2.1 Introduction	[line 287 after bullet till line 288 before bullet, delete and replace text:] "In this case, the plant has to be assessed in regard to the process of the modification (see point 3.1.3) and the changes introduced at the target sequence(s) (see point 3.1.2). In general, the extent of specific on-target and off-target effects of SDN-1 and SDN-2 (and also SDN-3) interventions largely depends on various experimental parameters such as: (i) the specific nuclease(s) used; (ii) the target organism and its tissue, respectively; (iii) the targeted gene(s); (iv) the way in which the components are introduced into the cells; (v) the dosage of the nuclease(s); (vi) with CRISPR/Cas, the guide RNA used and (vii) duration of the intervention (for overview, see Agapito-Tenfen et al., 2018; Eckerstorfer et al., 2019, Kawall et al., 2020). All these technical details determine the precision as well as the efficiency of an intervention. They need to be taken into account by competent authorities in order to identify potential unintended effects specifically caused by a specific genome editing intervention."	The GMO Panel considers that for the comments related to lines 287-288 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.	156
Sciensano	3.2.1 Introduction	Line 285-289 : particularly for scenario 2 (SDN module and any other exogenous DNA sequence is not present in plant genome) : the ToR should include an assessment of any potential additional risks for human health and the environment as compared to those of plants obtained with classic mutagenesis techniques.	The GMO Panel would like to clarify that the ToR have been provided by the European Commission and the panel has developed an opinion by strictly adhering to them. The GMO Panel was not mandated to develop new risk assessment guidelines for plants developed via genome editing approaches.	157
• Cornell University's Alliance for Science	3.2.1 Introduction	For the first scenario described by The Panel (with exogenous DNA), in Section 3.2.1, line 279, The Panel, states that the final products obtained via SDN-1, SDN-2, and ODM methods with exogenous DNA should be risk assessed as a transgenic plant "with regards to the exogenous DNA integrated in the genome" and as a gene-edited plant "in relation to the target sequence(s) which was modified via SDN-1, SDN-2, or ODM approaches". What this means and how it would be applied is not clear and needs elaboration. In particular: o We believe clarity is needed on what constitutes a "gene-edited plant"? Is this a new specific category that EFSA will establish? If so, how does the panel define the category? o We believe The Panel needs to explain the assessments that should apply "in relation to the target sequence(s) which was modified via SDN-1, SDN-2, or ODM approaches"; will the assessments focus on the phenotype or Genotype? And, to what extent? For the second scenario (without exogenous DNA), described in Section 3.2.1 line 284 The Panel states that those plants will only be assessed with regards to the modification introduced at the target sequence(s). Additional clarity is needed to understand what the GMO Panel means in this Section and how they would operate in practice. In particular:	The GMO Panel was mandated neither to provide a definition of genome edited plants nor to develop new guidances for their risk assessment which is still performed under the current EU regulation of GMOs (CJEU in Case C-528/16). According to the current regulation, the risk assessment will cover both molecular characterization (genotype) and food&feed/environmental assessment (phenotype). The GMO Panel considered important to provide just an operational distinction between these two scenarios in order to establish the	158

		o It would be helpful for all stakeholders if the panel clarified if the phrase "the target sequence(s) which was modified via SDN-1, SDN-2, or ODM approaches" stated in line 284 is intended to mean the same as the phrase used by the panel in line 288 when stating "the modification introduced at the target sequence(s)". o Moreover, we would like the panel to expand on the assessments that will be applied "with regards to the modification introduced at the target sequence(s)" via SDN-1, SDN-2, or ODM approaches". Will it be limited to the novelty of the trait and focus on phenotypic changes (as described in Section 3.2.2.1) or will the assessments also take into account genotype and, if so, to what extent?	background for the following sections. Since genome editing techniques can target theoretically any loci in the genome, the expression "target sequence" refers to any genomic nucleotide sequence intended to be modified. Nevertheless, some text refinements have been introduced to improve clarity.	
GenØk-centre for biosafety	3.2.1 Introduction	For details regarding this section: please read our attached table with our comments. **Copied from the submitted pdf file:** EFSA focuses on the final product not the techniques used Differently to what has been requested by the EC in its ToRs, EFSA focuses its assessment on two scenarios described by its final products. In other words, it is not possible to determine which techniques and supporting techniques are being applied in both scenarios. EFSA does not fulfills its mandate as it does not provide an assessment of the techniques but rather on fictitious products that could be obtained by many and different techniques of genetic engineering. For example, in lines 281 and 282, EFSA describes scenario #1 as "the full SDN module, part of it, or any exogenous DNA sequence deployed during the genome editing process is present in the plant genome." These products could be obtained by techniques of transgenesis, SDN-2, SDN-3 or ODM. How can EFSA assess the risks of such organism if the techniques applied are not described? How can EFSA assess the risks if it is not described whether a nuclease, a plasmid or an oligonucleotide molecule has been inserted in the cell? The evidence for such flawed assessment is provide in the following sections.	Section 3.2.1 is meant to provide an introductory part to the assessment of the applicability of the section 4 of the EFSA opinion on SDN-3 to plant developed by SDN-1, SDN-2, and ODM. Clearly, the most obvious difference between SDN-3 and the other techniques is that with the latter ones no exogenous DNA is meant to be integrated in the plant genome. However, the GMO Panel considers that in some cases exogenous DNA could be integrated for different reasons. The GMO Panel was not mandated to provide a detailed description of all the techniques used to achieve SDN-1, SDN-2 or ODM type of intervention. For this reason, the GMO Panel focused the assessment of the applicability of section 4 of the EFSA opinion on SDN-3 on the type of modification (i.e. SDN-1, SDN-2 and ODM) rather than the specific technology used to achieve such modification.	159
Federal Agency for Nature Conservation	3.2.1 Introduction	Lines 285-288: In case the SDN module and any other exogenous DNA sequences that were used during the SDN intervention are absent from the final product, the assessment should not be restricted to the modification at the target site, but include the identification and analysis of on-target and off-target changes. Their extent depends on a number of variables (see comment to lines 329-330).	Regarding the comment for line 285-288, the text has been revised to improve clarity. The GMO Panel invites also the contributor to refer to the responses given for the comments related to section 3.2.2 and its sub-sections for the off-target and on-target changes.	160

Envirnonmental association Za Zemiata	3.2.1 Introduction	[line 287 after bullet till line 288 before bullet, delete and replace text:] "In this case, the plant has to be assessed in regard to the process of the modification (see point 3.1.3) and the changes introduced at the target sequence(s) (see point 3.1.2). In general, the extent of specific on-target and off-target effects of SDN-1 and SDN-2 (and also SDN-3) interventions largely depends on various experimental parameters such as: (i) the specific nuclease(s) used; (ii) the target organism and its tissue, respectively; (iii) the targeted gene(s); (iv) the way in which the components are introduced into the cells; (v) the dosage of the nuclease(s); (vi) with CRISPR/Cas, the guide RNA used and (vii) duration of the intervention (for overview, see Agapito-Tenfen et al., 2018; Eckerstorfer et al., 2019). All these technical details determine the precision as well as the efficiency of an intervention. They need to be taken into account by competent authorities in order to identify potential unintended effects specifically caused by a specific genome editing intervention."	The GMO Panel considers that for the comments related to lines 287-288 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. Moreover, the points raised in the comment have been taken into account in section 3.2.2.	161
National Food Institute, Technical University of Denmark	3.2.2 Assessment of Section 4 of the EFSA opinion on SDN 3 - no text	The outcome of using SDN-1/SDN-2 would benefit from a direct comparison with the outcome of conventional mutagenesis, where no specific data and no risk assessment is needed. This would also be relevant for many traditional genetic modifications where the inserted gene is very similar to already existing genes in the host organisms and the effects of the insertion of the gene is expected to be comparable to traditional breeding. Decades of experience have shown this to be true and stated that the strict regulation and the scientific data requirement in EU (based on EFSA guidelines) in relation to GMO applications have not been and are still not justified.	The GMO Panel thanks for the comment. The EFSA opinion on SDN-3 was developed by comparing the type of outcome and mutations produced by SDN-3 to those generated by conventional breeding, including random mutagenesis. For this reason, the GMO Panel followed the same approach for SDN-1, SDN-2, and ODM, in order to be able to assess the applicability of section 4 and conclusions of that opinion to plant developed via these approaches.	162
CropLife Canada	3.2.2 Assessment of Section 4 of the EFSA opinion on SDN 3 - no text	-Lines 306-308: CropLife Canada agrees with the GMO panel conclusion that section 4.1 of the EFSA opinion on SDN3 is applicable only in parts to plants developed by SDN1, SDN2 and ODM approaches. Since there is no insertion of a transgene but rather a modification of an already existing endogenous sequence, the risk assessment process should heavily rely on history of safe use, the fact that novel toxins or allergens have never been introduced through conventional breeding, and plant breeders routinely measure levels of known toxins and allergens throughout the breeding process. This could be easily established with the application of the problem formulation approach, and a brief justification can be provided to address this section, thus avoiding the need to revise the existing EFSA guidance. In addition to early consultation with product developers, CropLife Canada believes that it would be valuable if EFSA could identify and provide a list of studies, currently part of the guidance and which focus on the transgene that are potentially not applicable – e.g. gene expression, bioinformatics of the transgene sequence, protein allergenicity and toxicity studies, etc. CropLife Canada is of the view that it would be beneficial both for EFSA and applicants if the guidance around SDN-1, SDN-2 and ODM products is aligned with that of other jurisdictions -Lines 322 – 324: CropLife Canada agrees with the conclusion of the EFSA GMO panel in this section. As there is no insertion, this section does not apply. This could be easily established with the application of the problem formulation approach, and a brief justification can be provided to address this section, thus avoiding the need to revise the existing EFSA guidance. Lines 352 – 354: CropLife Canada agrees that providing the analysis of potential off-target effects is of limited value for plants developed using SDN and ODM approaches given their targeted nature. In	The GMO Panel thanks CropLife Canada for the comment and takes note of it. Depending on the methods which was used to generate the genome edited plant and the traits characterizing such products, the GMO panel may consider some data requirements not necessary for the risk assessment. The GMO Panel considers that the "case-by-case" approach as described in the opinion on SDN-3 is also applicable to genome edited plants. This position is in line with the conclusions of the opinion stating that the EFSA guidances are sufficient but can be only partially applied for the risk assessment of plants generated by the	163

		the final report, it would be of benefit for concerned stakeholders if EFSA could identify here a list of example studies that are currently part of the guidance which are solely focused on off-target analysis and that do not apply to these technologies. For instance, general untargeted comparative compositional and agronomic analysis often used to confirm the lack of unintended effects should not apply to SDN-1, SDN-2 and ODM given that their targeted nature produce changes similar to what can occur in conventional breeding, which has been used in thousands of varieties with no cases of toxins or allergens introduced.	application of SDN-1, SDN-2, and ODM methods, especially when a transgene is not present in the final product. Moreover, the GMO Panel was not mandated to provide a comprehensive list of the studies required or not for the risk assessment of genome edited plants.	
ENSSER	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	L292: As transgenesis is often used or may accidentally occur as a step in SDN-1 and SDN-2, the first sentence requires more clarity. It is also missing a major aspect of SDN-3, namely the insertion of any gene sequence irrespective of its origin. We suggest the following ammendment: "SDN-1, SDN-2, and ODM approaches differ from SDN-3 and transgenesis in that their action is not intended to result in the insertion of any transgene or any longer DNA sequence but rather in the modification of an already existing endogenous sequence." L296: This statement lacks clarity and gives the wrong impression that a "history of safe use and consumption" of a trait/gene/compound is considerted as a green light for its use in a different context. Please correct the sentence accordingly, and add the following: "recognising that the final product might be different from that with a history of safe use and consumption (e.g. different glycosilation patterns), or that due to the presence of this substance or trait other substances or traits or metabolic pathways might be affected." This should especially be considered when a trait is out of or in a different context (Prescott et al. 2005). This also has to bear in mind that due to SDN-1 based modifications there may be truncations or frame-shifts that may give rise to new RNA molecules, polypeptides or even functional proteins (Tuladhar et al. 2019) L300: concerning "and specific data on the edited gene may not be needed" What is missing missing here is the assessment of unintended effects that need to be considered when genome editing was applied. L304: concerning "in some cases only a subset of the data required for SDN-3 would be needed" Which subset(s) is(are) being referred to or thought of here? And what about additional data required? As stated previously, there are additonal risks arising from some SDN-1 applications due added uncertainties, complexities, off-target alterations, altered or 'foreign' mRNAs (Tulahadhar et al. 2019), truncated proteins, etc. This needs adressing and not avoiding. Thus please add the following after "would be needed": "whilst additional information or subsets may also be needed." L306: Unfortunately, instead of investigating risk scenarios and risk hypothesis further and expanding	Regarding comment for line 292, the expression "or any DNA sequence" has been inserted in the text to improve clarity. Regarding comment for line 296, the GMO Panel acknowledges that the risk assessment process will not be limited only to considerations related to the history of safe use and consumption. The text has been revised accordingly. Regarding comment for line 300, the GMO Panel invites the contributor to refer to the responses given for the comments related to section 3.2.2 and its sub-sections for the off-target and on-target changes. Regarding comment to line 304, the GMO Panel considers that the sentence correctly reflects the conclusion of the opinion stating that the guidance are sufficient but on a case-by-case basis a subset of the data may be needed, depending on the trait and the type of product. Regarding comment for line 306, the GMO Panel would like to clarify that section 3.2.2.1 and its conclusions refer to the assessment of the applicability of	164

		requirements, the panel has chosen to reducing requirements. We would like this opinion to state clearly what "in part" means and what the consequences are. And what is to be done about the additional risks that in fact create substances and pathways -intentionally or unintentionally- that were not present prior to the modifications. It needs to be acknowledged that SDN-1 and -2 have completely different aims as compared to SDN-3, and that a very different repair mechanism is active during SDN-1 activity. The comparitability of SDN-1 and SDN-3 is very limited due to their clear differences. In brief: whilst in may be "in parts applicable", but there is a vast section missing in this statement and it is thus insufficient.	section 4.1 of the opinion on SDN-3. The partial applicability of that section is due to the absence of any inserted DNA sequence at the target site(s), hence all the considerations related to the presence of an inserted sequence are not applicable to plants developed via SDN-1, SDN-2, and ODM (please, see also the conclusions of the draft opinion).	
EuropaBio	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	Lines 292-308. In this section EFSA describes possible extreme editing examples and acknowledges that there will be variations within, concluding that section 4.1 of the EFSA opinion on SDN-3 is applicable "only in part". EuropaBio considers that EFSA should clarify here that applying the problem formulation approach will guide the risk assessment and help establish what specific data on the new allele and expressed trait may be relevant for a given product. Where the edits characterizing the final product are already present in a consumed variety of the same species we agree with the EFSA position that specific data on the edited gene(s) may not be needed. Existing EFSA guidance may not be applicable or relevant in some cases, but this does not imply a need for the revision of current guidance. We consider that the principle of proportionality as set out in Article 5 of Regulation (EC) No178/2002 should be mentioned here.	The GMO Panel takes note of the comment. According to the judgement of the Court of Justice of the European Union (CJEU) in Case C-528/16, Directive 2001/18 is applicable to genome edited plants which are considered GMOs within the meaning of that directive. For this reason, the panel would perform the risk assessment of genome edited plants according to all the provisions laid down in the EU regulation of GMOs.	165
Association Française de Biotechnologies Végétales	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	AFBV edits and comments: Line 293: After the word "transgene" insert ", intragene or cisgene" consistently with Lines 156 and 236. Line 296: Replace the word "extreme" with "likely". Line 297: Replace "On one extreme," with "In one case" Line 300: Replace "On the other extreme, " with "In another case" Line 303: After "assessment" insert "if the modification achieved is one that could not occur through a relevant conventional breeding technique." To avoid confusion and misinterpretation, it would probably be better not to use the term "extreme" since the two examples provided are expected and do not raise any safety concerns. The insertion at line 303 has been added because new, never-before-seen mutations obtained by conventional breeding techniques are common and raise no concerns. The types of mutations achieved by genome editing techniques are similar to those obtained by conventional breeding techniques and should raise a concern for additional data only if the type of mutation could not be achievable	Regarding comment for line 293, the term "transgene" has been replaced by the term "DNA sequence". Regarding comments for lines 296, 297, and 300, please note that the text has been revised to improve clarity. Regarding comment for line 303, according to the judgement of the Court of Justice of the European Union (CJEU) in Case C-528/16, Directive 2001/18 is applicable to genome edited plants which are considered GMOs within the meaning of that directive. For this reason, the panel would perform the risk assessment of genome edited plants according to all the	166

		through conventional breeding techniques. AFBV Proposal for certain Genome-Edited Plants. As mentioned in our comments under Section 2.1.3. above, in the proposal sent by AFBV to the Commission in February we suggested that four categories of edited plants be excluded from the GMO legislation: (1) plants having a native allele that has been edited to reproduce a functionality associated with a known allele present in its natural gene pool; (2) plants having a native allele that has been edited to reproduce a functionality associated with a known allele present in a plant species that is outside the plant's natural gene pool; (3) plants having a native allele that has been edited to reproduce a new functionality, of which the sequence modifications obtained by genome editing are of the same type as those which be obtained by spontaneous or induced mutagenesis; and (4) plants possessing a gene known and present in its natural gene pool which has been inserted into a targeted site of its genome. AFBV defined "natural gene pool" as follows: to the gene pool of a plant species defined as all of the genes and alleles (i.e., different versions of the same gene) obtained from plants which can exchange genes by sexual crossing as well as from distantly related plant species with which genes can be exchanged by sexual crosses using traditional breeding techniques. Plants from AFBV's category 1 would include plants described by EFSA in its first case; those of category 3 could fit with EFSA's second case and AFBV's category 2 could correspond to a case in-between EFSA's two cases. Category 4 corresponds to the insertion of a cisgene using SDN-3 technique (see AFBV comment at the end of Section 2.1.3). AFBV proposed that the four above categories of edited plants be excluded from the application of GMO legislation on a case-by-case basis after a confirmation process whereby the applicant would provide sufficient information to the competent authority of a member state to confirm the excluded status of the edited plant. Amongst the information to be provided would be the origin and description of the new allele and confirmation of the absence of exogenous DNA. If a transgene is used to perform the edit, it should be removed from the edited plants. Such excluded edited plants would follow the regulations which apply for varieties derived from conventional breeding. (See enclosed Explanatory Note and Draft Amendment to Directive 2001/18 for further details.) Our proposal is in line with EFSA's request regarding the need "on a case-by-case basis" for "lesser amounts of event-specific data" needed for the "risk assessment of plants developed using the SDN-3 technique…. and therefore a need for flexibility in the data requirements for risk assessments…"	provisions laid down in the EU regulation of GMOs. The fact that the specific allele/trait can also be theoretically achievable by mean of conventional breeding approaches is not relevant according to the EU regulation in place (meaning, this product cannot be exempted from the risk assessment process in case it was developed via genome editing approaches). The GMO Panel takes note of the remaining comments.	
Federal Office of Consumer Protection and Food Safety (BVL), Competent Authority according to Directive 2001/18/EC	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	Line 302: We suggest to stick to the phrasing in Line 301 and use the term "associated trait" instead of "expressed trait" to not mix up with molecular terminology (in general, proteins are expressed from genes, not traits). Line 301-305: Various plant breeding approaches using SDN-1 or SDN-2 make use of multiparallel site specific mutagenesis strategies, the so called multiplexing. A statement here or in Section 3.3 on the applicability of EFSA's SDN3 opinion on those products displaying an accumulation of site specific mutations by multiplexing is missing. Alternatively, a reasoning should be presented, why SDN-1 and SDN-2 multiplexed plants are not within the terms of references.	Regarding comment for line 302, the text has been amended accordingly. Regarding comment for lines 301-305, the GMO Panel understands that the term "multiplexing" used in the comment may refer to the simultaneous mutation of multiple plant genomic loci. Although	167

			multiplexing approach is not specifically discussed in the opinion, the GMO Panel considers that all the considerations included in the opinion on SDN-based methods are also applicable to multiplexing approaches. Moreover, it should be noted that multiplexing is not specific to SDN/ODM approaches as it can also be achieved by transgenic and conventional breeding approaches. The GMO Panel would also like to remind that the "case-by-case" approach can also be applied to genome edited plants. The GMO Panel knows that a complexity of scenarios is possible due to the application of SDN-based methods. In this regard, the GMO Panel refers to the mandate on GM plant generated via synthetic biology approaches.	
Euroseeds	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	The EFSA GMO Panel correctly identified the two extreme cases and the continuum between them. It should be noted that for conventional breeding (including wide crosses and mutation breeding) also the second case of a new trait occurs. Breeders and regulators have for decades successfully put into practice approaches that ensure the safety of new varieties. Therefore, in view of the principle of proportionality, we consider that only in rare cases a traditionally bred crop with a new trait/allele (as to line 302) would trigger the application of Regulation EU 2015/2283 for novel foods. The same applies to additional specific data that would be necessary to risk assess the crop developed with targeted mutagenesis approaches (9) . In all other cases a problem formulation can determine if the same elements as those evaluated for conventionally bred plants are sufficient to assess the organism developed using targeted mutagenesis approaches. In case of specific crops this might include the evaluation of the level of specific compounds. Lines 307/323: we would prefer not applicable AND not RELEVANT to avoid the impression that new guidance in this context is needed. (9) "The Novel Food Regulation will apply to conventional food if it consists of or is isolated or produced from a plant or a variety of the same species obtained by non-traditional propagating practices that give rise to significant changes in the composition or structure of the food affecting its nutritional value, metabolism or level of undesirable substances. If the Novel Food Regulation conditions apply, the food in question will be subject to a GMO-like pre-market risk assessment and authorisation." FRom farm to fork: the regulatory status of non-GMO plant innovations under current EU law, Bioscience Law Rev. VOL 16 ISSUE 6 (2018)	The GMO Panel thanks for the comment and takes note of it. Regarding comment for lines 307/323, the GMO Panel notes that the term "applicable" is the same as the one used in the terms of reference provided by the European Commission.	168

Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG)	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	line 300: unclesr the meaning of the "other extreme"	Please note that the text has been revised to improve clarity.	169
Union Française des Semenciers	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	UFS emphasizes that, even for traditional breeding (including wide crosses and mutation breeding), the case of a new undescribed trait occurs. Since decades, breeders are used to carrying out practice approaches to ensure the safety of new varieties. Consequently, as regards the principle of proportionality, only in rare cases a traditionally bred crop with a new trait/allele (as to line 302) might trigger the application of Regulation EU 2015/2283 on Novel food. The same applies to additional specific data that would be necessary to assess the risk brought by a variety developed by targeted mutagenesis (1). In all other cases a problem formulation can determine if the same elements as those evaluated for conventionally bred plants are sufficient to assess the organism developed using targeted mutagenesis approaches. In case of specific crops this might include the evaluation of the level of specific compounds. - Line 323: In line with all those considerations and explanations, UFS would prefer "is not applicable and not relevant" to avoid the impression that new guidance in this context is needed. This is also in line with Conclusion (line 424). (1) The Novel Food Regulation will apply to conventional food if it consists of or is isolated or produced from a plant or a variety of the same species obtained by non-traditional propagating practices that give rise to significant changes in the composition or structure of the food affecting its nutritional value, metabolism or level of undesirable substances. If the Novel Food Regulation conditions apply, the food in question will be subject to a GMO-like pre-market risk assessment and authorisation."	The GMO Panel thanks UFS and takes note of the comment. Regarding comment for line 323, the GMO Panel notes that the term "applicable" is the same as the one used in the terms of reference provided by the European Commission.	170
Plant Biotechnology Society	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	see attached file	The GMO Panel took note of the comment.	171
Scientific Committee for GM food and Feed, Advisory Body, Czech Republic	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	It is concuded that "in some cases only a subset of the data required for SDN-3 would be needed" for safety assessment of SDN-1, SDN-2, and ODM. Wording and meaning should be carefully checked However, taking into account the diametrical difference between the insertion of a stretch of DNA (from any source) in SDN-3 and the local modification of plants' own DNA, "only a subset of the data" should be required in all cases (not only "in some"). We suggest to check the meaning and improve the wording in the text.	The GMO Panel takes note of the comment. After the revision of the document, the GMO Panel considers the conclusions still valid.	172

GMO Office, National Institute of Public Health and the Environment (RIVM)	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	Line 297-300 Given the examples described in the opinion, a 'history of safe use' seems only related to prior consumption of the crop. However, since this opinion also deals with the environmental risk assessment of plants (that may not always be consumed by humans), it is suggested to extend the history of safe use also to crops that have been prior cultivated.	The GMO Panel thanks RIVM for the comment. The text has been revised to extend the notion of history of safe use also to the environmental risk assessment.	173
National Food Institute, Technical University of Denmark	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	The extreme describing a modified allele that has never been described before is not unique to SDN-1/SDN-2, this is what is achieved all the time with conventional mutagenesis as well.	The GMO Panel takes note of the comment. According to the judgement of the Court of Justice of the European Union (CJEU) in Case C-528/16, Directive 2001/18 is applicable to genome edited plants which are considered GMOs within the meaning of that directive. For this reason, the panel would perform the risk assessment of genome edited plants according to all the provisions laid down in the EU regulation of GMOs. The fact that the specific allele/trait can also be theoretically achievable by mean of conventional mutagenesis approaches is not relevant according to the EU regulation in place (meaning, this product cannot be exempted from the risk assessment process in case it was developed via genome editing approaches)	174
Plantum - Netherlands seed association	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	The report identifies two extreme cases and concludes that also in conventional breeding such range of cases could occur. This supports the conclusion that hazards are not additional to those in conventional breeding.	The GMO Panel takes note of the comment.	175
French agency for Food, Environmental and Occupational Health & Safety (Anses)	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	Page 9, lines 300-301: "On the other extreme, the modified allele and associated trait present in the final product have never been described before.": would it be relevant to consider this allele as exogenous DNA?	The operational definition of "exogenous DNA" used in the opinion is derived from the SAM report on New techniques in agricultural biotechnology (2017) which defines it as "DNA originating outside the plant which can be introduced naturally or by	176

			technological intervention". According to this definition, the modified allele cannot be considered exogenous DNA since no DNA originating outside the plant is introduced in the plant genome.	
Corteva Agriscience	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	Two extreme cases and the continuing between them were described by the EFSA GMO Panel. It should be noted that for conventional breeding (including wide crosses and mutation breeding) also the second case of a new trait occur. Breeders and regulators have for decades put into practice approaches that ensure safety of new varieties. Therefore, considering the principle of proportionality, we would consider that only in the most extreme cases where also for a traditionally bred crop with a new trait that would trigger the application of Regulation EU 2015/2283 for novel foods, additional specific data would be necessary to risk assess the crop developed with SDN techniques. In all other cases the same elements as those evaluated for conventional bred crops should be sufficient to assess the introduced trait. In case of specific crops this might include the evaluation of the level of specific compounds.	The GMO Panel thanks Corteva Agriscience and takes note of the comment.	177
Haut Conseil des biotechnologies (High Council for Biotechnology)	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	l. 294-296. "Depending on the nature of the gene/locus modified and the origin of the allele and trait associated with the final product, the risk assessment process will necessarily take into consideration the history of safe use and consumption." We suggest completing the end of the sentence for clarity as follows: "the history of safe use and consumption of food/feed from potential plants known to express the modified allele and trait associated with the final product.". It seems that in any case, the new allele and the expressed trait will have to be considered. The risk assessment could follow a precise decision-tree type of procedure, whereby, depending on the cases, different types and amounts of data will have to be provided by the applicant.	The text has been revised to include within the history of safe use concept both the areas of food&feed and the environment.	178
Testbiotech	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	[Line 300 after first bullet add further text:] "In addition, the steps of the processes involved to achieve the modification (see 3.1.4) still have to be considered." [Line 303 after first bullet, till line 308 delete and replace text:] "The GMO Panel considers that a substantial number of different scenarios are possible between these two extremes. Each of them will require a set of data concerning the different steps of the process (see point 3.1.4). The set of data might deviate from those as requested for SDN-3 and also might go beyond. For example, if an applicant has to show that the new allele obtained through genome editing and the associated trait characterizing the final product are already present in a consumed variety of the same species, whole genome sequencing might be requested, also for the identification of unintended effects that are generated during the transformation process and/or the biological mutagens. On the other hand, if the newly generated gene combination (see point 3.1.2) results in profound changes, for example, of the plant metabolism, the comparative risk assessment may be challenged to an extent that goes beyond the existing experience with transgenic plants or SDN-3 applications.	The GMO Panel considers that the assessment of genome edited plants follows the regulation for GMOs currently in place in the European Union. The assessment takes already into consideration the methodology used to generate the product. Regarding the identification of unintended effects, the GMO Panel invites Testbiotech to refer to the responses given for the comments related to section 3.2.2.2 and its sub-sections. The GMO Panel acknowledges that the choice of comparator could be	179

		Under such circumstances, new methods for risk assessment, such as metabolomics, proteomics and transcriptomics, might be needed to perform risk assessment in the absence of adequate comparators. In regard to environmental risk assessment, there are several risk scenarios that need to be considered, such as changes in the composition of plants that may impact the food web, changes in the composition of plants that may impact plant communication and interaction with the environment, changes in the biological characteristics of the plants that concern their invasiveness and next generation effects of plants with the potential to persist and propagate in the environment (for overview see Testbiotech (2020); see also Bauer-Panskus et al., 2020). There are several publications showing a broad range of aspects that have to be taken into account in regard to the safety of gene products and organisms derived from SDN-1 and SDN-2 processes (for overview, see Agapito-Tenfen et al., 2018, Eckerstorfer et al., 2019; Testbiotech , 2020, Cotter et al., 2020; Kawall et al., 2020). Because of the abovementioned considerations, the GMO Panel concludes that the section 4.1 of the EFSA opinion on SDN3 ("Source of genes and safety of gene products") is partially applicable, but will in many cases not be sufficient to assess the risks of plants developed by SDN-1, SDN-2. Since in regard to ODM relevant data are mostly missing, no final conclusion can be derived."	more difficult in case of complex (e.g. polygenic) traits associated to the edited plant. However, it should be noted that multigene modifications leading to the alteration of existing traits or the generation of new complex ones, including e.g. modification of plant metabolism affecting multiple signaling pathways and having relevance for the interaction with the environment, could also be achieved by conventional breeding and traditional transgenesis; hence, this is neither a novel scenario nor a new hazard which is limited only to genome edited plants. The GMO Panel would also like to remind that the "case-by-case" approach as described in the opinion on SDN 3 is also applicable to genome edited plants.	
Umweltbundesamt (Environment Agency Austria) on behalf of the Austrian lead Competent Authority, the Federal Ministry of Social Affairs, Health, Care and Consumer Protection.	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	Lines 296ff: EFSA differentiates 2 "extreme" cases for discussion. We note that in a recent publication that was presented to the EFSA Scientific Network meeting in 2019 actually 3 different general categories are described (Eckerstorfer et al., 2019a). Category (1) and (3) are matching in essence with the extreme cases outlined in the draft opinion. We recommend to include the third category (see below*) from the paper as well, as we consider this case relevant and helpful for structuring a case-specific risk assessment. In any case, we are of the opinion that the mode of action of the trait, the resulting changes in the genotype, the phenotype and the metabolism of the plant should be assessed based on available or newly established experimental data (EFSA 2011). Hence, the provisions of EFSA (EFSA 2011, 3.1.2.2) should be followed. This comprises provisions regarding the evidence of intended changes of the modified sequences by analysis of specific DNA, changes of RNA(s), metabolite(s) or protein levels and the comparison with already existing varieties with similar traits. These data are necessary to ensure that the intended modification is effective and that potential unintended effects are detected (e.g. due to different outcomes of repair at the double strand breaks or due to the gene delivery into the plant cell). These data should be derived from plants grown under representative growing conditions. In order to establish the comparability and equivalence with an existing variety, the experimental design could include such an existing variety as a non-GM comparator in order to compare the levels of relevant endogenous RNA(s), protein(s) and/or specific metabolite(s) (see EFSA 2011). * (omitted) Category (2): Genome edited (nGM) plants with traits similar to those established in GM plants, e.g., herbicide resistance, disease resistance or insecticidal traits. For this category of nGM plants similar approaches for risk assessment to those implemented for the respective GMOs should be applied. Previous experiences with the assessment of such GMOs should be taken into account for	Regarding comment for line 296, the text of the section 3.2.2.1 includes already the category 2 describe in the comment. This category would fall between the 2 different scenarios already described in the text. The GMO Panel reminds that according to the judgement of the Court of Justice of the European Union (CJEU) in Case C-528/16, Directive 2001/18 is applicable to genome edited plants which are considered GMOs within the meaning of that directive. For this reason, the panel would perform the risk assessment of genome edited plants according to all the provisions laid down in the EU regulation of GMOs. However, as stated in this opinion, the GMO Panel would follow the "case-by-case" approach given the variety of products achievable by the	180

		the development of risk assessment approaches specifically adapted to the characteristics of nGM plants. Line 307f: The draft opinion concludes that the section 4.1 of the EFSA opinion on SDN3 "is applicable only in parts" and that for SDN-1, SDN-2 and ODM "in some cases only a subset of data required for SDN-3 would be needed" (c.f. Line 304). Based on examples provided in the revised opinion it should be detailed which subset of data would be considered necessary. Eckerstorfer, Michael F.; Dolezel, Marion; Heissenberger, Andreas; Miklau, Marianne; Reichenbecher, Wolfram; Steinbrecher, Ricarda A.; Waßmann, Friedrich (2019a): An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs). Frontiers in bioengineering and bio-technology 7, S. 31. DOI: 10.3389/fbioe.2019.00031. EFSA (2011): EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011; 9(5): 2150. [37 pp.] doi:10.2903/j.efsa.2011.2150.	application of SDN1, SDN2, and ODM. The GMO Panel also concludes that a number of requirements of the existing guidances that are linked to the presence of a transgene are not relevant for the assessment of SDN1, SDN2 and ODM plants in case the final product does not contain any exogenous DNA. Regarding the comment for line 307, the GMO Panel was neither mandated to provide a comprehensive list of the requirements needed for the risk assessment nor to revise point-by-point the current guidances.	
V, Ganesh kumar	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	In Line number 302 of Page 9 it was mentioned that "specific data on the new allele and the expressed trait would be needed to perform the risk assessment" if the modified allele and associated trait present in the final product have never been described before. This is not required, most valuable contribution from genome editing will come from new/novel alleles rapidly generated using this technology (we can say, it's a kind of accelerating the mutation frequency). These kind of novel/new mutations are also created and utilized in conventional mutagenesis technologies and hence this requirement needs to be re-evaluated for elimination. Here, the emphasis should be given to ensure "whether the same mutation can be practically achieved through traditional mutagenesis method also". If the answer is yes, then specific data requirement is not necessary. If the answer is no, then the data can be made necessary.	Regarding comment for line 302, according to the judgement of the Court of Justice of the European Union (CJEU) in Case C-528/16, Directive 2001/18 is applicable to genome edited plants which are considered GMOs within the meaning of that directive. For this reason, the panel would perform the risk assessment of genome edited plants according to all the provisions laid down in the EU regulation of GMOs. The fact that the specific allele/trait can also be theoretically achievable by mean of traditional mutagenesis methods is not relevant according to the EU regulation in place (meaning, this product cannot be exempted from the risk assessment process in case it was developed via genome editing approaches).	181
Sciensano	3.2.2.1 Assessment of section 4.1: Source of genes and	It emphasizes that an assessment of any potential additional risks for human health and the environment as compared to those of plants obtained with classic mutagenesis techniques has been totally neglected.	The GMO Panel takes note of the comment.	182

	safety of gene products			
Società Italiana di Genetica Agraria - Italian Society of Agricultural Genetics (SIGA)	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	Lines 314-315 "… do not induce DSB in the plant genome at any stage during the process". We suggest to specify that a single strand break is necessary to trigger prime editing: "… do not induce DSB in the plant genome at any stage during the process, but they rather induce a single strand break at the target site".	The text has been amended accordingly.	183
Cornell University's Alliance for Science	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	• We believe The Panel correctly used the distinctions made in Section "3.1.3 Methods for delivering or expressing SDN in plants" when evaluating the applicability of "Section 4" and the "Conclusions" of the EFSA opinion on SDN-3 to plants developed using SDN-1, SDN-2, and ODM techniques. And in general, The Panel correctly concludes that Section 4.1 of the EFSA opinion on SDN3 (Source of genes and safety of gene products) is applicable only in part to plants developed by SDN1, SDN2, and ODM approaches. • The analysis made by the panel in Section 3.2.2.1 is correct in finding that the risk assessment needed could vary depending on the nature of the gene/locus modified and the origin of the allele and trait associated with the final product. However, we believe stakeholders would benefit from obtaining more clarity on The Panels statement in line 303 and what they recommend should be done to address it. The Panel states in "Section 3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products", line 303, that there is a substantial number of different scenarios possible between the two extremes presented from line 294 to 302. Stakeholders would benefit from: a) more examples of what these in-between scenarios could be? and b) how EFSA would decide what the different risk assessment requirements would apply? • In general we agree with The GMO Panel correctly concluding that the section 4.1 of the EFSA opinion on SDN3 ("Source of genes and safety of gene products") is applicable only in part to plants developed by SDN1, SDN2, and ODM approaches.	The GMO Panel thanks for the comments.	184
GenØk-centre for biosafety	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	SDN-1, SDN-2 and ODM techniques differ from SDN-3 as they do not include an insertion of transgenic sequences. The GMO panel thus considers that section 4.1 of the EFSA opinion on SDN-3 can be used/applied in part to plants developed by the other techniques (SDN-1, SDN-2 and ODM). In the risk assessment they consider two scenarios ;1:new allelle already consumed and history of safe use can be used, 2: new undescribed trait needing specific data. The repair mechanisms is not mentioned in this section of the assessment and should be mentioned to highlight that although these mechanisms are known, they vary between cell types and there is still much that is unknown about how distinct cell types work. Cas-9 proteins and other editing enzymes have the potential to create genomic instability in cases where polymerases and helicases are disrupted. These enzymes are part of the DNA replication and transcription machinery. Cells do repair these errors as well. These non-target changes in the genome is not mentioned in the draft part here, but should have a note with a focus on choice of editing system, specificity of repair mechanisms, and analysis of potential off-target effects.	In section 3.2.2.1, the GMO Panel assessed the applicability of section 4.1 of the EFSA opinion on SDN3 to plant obtained via SDN1, SDN2, and ODM. Section 4.1 of that opinion does not address the "non target changes" of site directed nucleases. Regarding this topic, the GMO Panel invites the contributor to refer to section 3.2.2.2 and the responses to the comments related to that section.	185

		For additional details regarding this section: please read our attached table with our comments. **Copied from the submitted pdf file:** The presence of foreign DNA should not be the only criteria for analysis EFSA states that "SDN-1, SDN-2 and ODM approaches differ from SDN-3 and transgenesis in that they do not result in the insertion of any transgene but rather in the modification of an already existing endogenous sequence." Whereas EFSA might be right that a transgene insertion is not expected in SDN-1, SDN-2 and ODM approaches, the integration of exogenous foreign DNA used either as a template or as part of a delivery method (viral vectors, etc) should be verified during RA when these approaches are used. In other words, it cannot be assumed that when using SDN-1, SDN-2 and ODM foreign DNA introgression is not present in the genome. This kind of assumption has lead to the discovery of plasmid sequences in the genome of gene-edited hornless cattle by the Food and Drug Administration Department in the U.S5. Neither the developer of the Brazilian Biosafety Authority (CTNBio), which granted non-GMO status to this organism, has detected foreign DNA sequences in the genome of the cattle. Both the company and CTNBio assumed that the non-integrative plasmid containing TALEN and DNA template plasmid sequences were not able to be inserted in the host genome and did not verified that.		
Envirnonmental association Za Zemiata	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	[Line 300 after first bullet add further text:] "In addition, the steps of the processes involved to achieve the modification (see 3.1.4) still have to be considered." [Line 303 after first bullet, till line 308 delete and replace text:] "The GMO Panel considers that a substantial number of different scenarios are possible between these two extremes. Each of them will require a set of data concerning the different steps of the process (see point 3.1.4). The set of data might deviate from those as requested for SDN-3 and also might go beyond. For example, if an applicant has to show that the new allele obtained through genome editing and the associated trait characterizing the final product are already present in a consumed variety of the same species, whole genome sequencing might be requested, also for the identification of unintended effects that are generated during the transformation process and/or the biological mutagens. On the other hand, if the newly generated gene combination (see point 3.1.2) results in profound changes, for example, of the plant metabolism, the comparative risk assessment may be challenged to an extent that goes beyond the existing experience with transgenic plants or SDN-3 applications. Under such circumstances, new methods for risk assessment, such as metabolomics, proteomics and transcriptomics, might be needed to perform risk assessment in the absence of adequate comparators.	The GMO Panel considers that the assessment of genome edited plants follows the regulation for GMOs currently in place in the European Union. The assessment takes already into consideration the methodology used to generate the product. Regarding the identification of unintended effects, the GMO Panel invites Testbiotech to refer to the responses given for the comments related to section 3.2.2.2 and its sub-sections. The GMO Panel acknowledges that the choice of comparator could be more difficult in case of complex (e.g. polygenic) traits associated to the edited plant. However, it should be noted that multigene	186

		In regard to environmental risk assessment, there are several risk scenarios that need to be considered, such as changes in the composition of plants that may impact the food web, changes in the composition of plants that may impact plant communication and interaction with the environment, changes in the biological characteristics of the plants that concern their invasiveness and next generation effects of plants with the potential to persist and propagate in the environment (for overview see Testbiotech (2020); see also Bauer-Panskus et al., 2020). There are several publications showing a broad range of aspects that have to be taken into account in regard to the safety of gene products and organisms derived from SDN-1 and SDN-2 processes (for overview, see Agapito-Tenfen et al., 2018, Eckerstorfer et al., 2019; Testbiotech , 2020, Cotter et al., 2020). Because of the abovementioned considerations, the GMO Panel concludes that the section 4.1 of the EFSA opinion on SDN3 ("Source of genes and safety of gene products") is partially applicable, but will in many cases not be sufficient to assess the risks of plants developed by SDN-1, SDN-2. Since in regard to ODM relevant data are mostly missing, no final conclusion can be derived."	modifications leading to the alteration of existing traits or the generation of new complex ones, including e.g. modification of plant metabolism affecting multiple signaling pathways and having relevance for the interaction with the environment, could also be achieved by conventional breeding and traditional transgenesis; hence, this is neither a novel scenario nor a new hazard which is limited only to genome edited plants. The GMO Panel would also like to remind that the "case-by-case" approach as described in the opinion on SDN 3 is also applicable to genome edited plants.	
BUND e.V. / Friends of the Earth Germany	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	Line 299 to 300 CHANGE sentence: "Still the risk assessment cannot only focus on the knowledge of the consumed variety (history of safe use) but specific data on the edited gene may still be needed, since the genomic pattern of the plant might be changed in comparison to known varieties obtained by conventional breeding, resulting in different specific risks (Kawall 2019)". Line 305 ADD after "needed": ", though in any case, a specific risk assesment for the genomic modification has to be applied (Eckerstorfer et al. 2019)" Line 308 ADD after "approaches": ", though this leaves unaffected the need for a specific risk assessment for SDN1, SDN2, and ODM approaches (Agapito-Tenfen et al. 2018)"	Regarding comment to lines 299-300, the GMO Panel considers that the information on the edited gene is part of the risk assessment. Please note that the "case-by-case" approach also applies to genome edited plants as it was for SDN-3. Regarding comments to line 305 and 308, the GMO Panel do not consider necessary to add the proposed sentences based on the response given for comment to lines 299-300.	187
CropLife Canada	3.2.2.1 Assessment of section 4.1: Source of genes and safety of gene products	-Lines 306-308: CropLife Canada agrees with the GMO panel conclusion that section 4.1 of the EFSA opinion on SDN3 is applicable only in parts to plants developed by SDN1, SDN2 and ODM approaches. Since there is no insertion of a transgene but rather a modification of an already existing endogenous sequence, the risk assessment process should heavily rely on history of safe use, the fact that novel toxins or allergens have never been introduced through conventional breeding, and plant breeders routinely measure levels of known toxins and allergens throughout the breeding process. This could be easily established with the application of the problem formulation approach, and a brief	The GMO Panel thanks CropLife Canada for the comment and takes note of it. Depending on the methods which was used to generate the genome edited plant and the traits characterizing such products, the GMO panel may	188

		justification can be provided to address this section, thus avoiding the need to revise the existing EFSA guidance. In addition to early consultation with product developers, CropLife Canada believes that it would be valuable if EFSA could identify and provide a list of studies, currently part of the guidance and which focus on the transgene that are potentially not applicable – e.g. gene expression, bioinformatics of the transgene sequence, protein allergenicity and toxicity studies, etc. CropLife Canada is of the view that it would be beneficial both for EFSA and applicants if the guidance around SDN-1, SDN-2 and ODM products is aligned with that of other jurisdictions -Lines 322 – 324: CropLife Canada agrees with the conclusion of the EFSA GMO panel in this section. As there is no insertion, this section does not apply. This could be easily established with the application of the problem formulation approach, and a brief justification can be provided to address this section, thus avoiding the need to revise the existing EFSA guidance. Lines 352 – 354: CropLife Canada agrees that providing the analysis of potential off-target effects is of limited value for plants developed using SDN and ODM approaches given their targeted nature. In the final report, it would be of benefit for concerned stakeholders if EFSA could identify here a list of example studies that are currently part of the guidance which are solely focused on off-target analysis and that do not apply to these technologies. For instance, general untargeted comparative compositional and agronomic analysis often used to confirm the lack of unintended effects should not apply to SDN-1, SDN-2 and ODM given that their targeted nature produce changes similar to what can occur in conventional breeding, which has been used in thousands of varieties with no cases of toxins or allergens introduced.	consider some data requirements not necessary for the risk assessment. The GMO Panel considers that the "case-by-case" approach as described in the opinion on SDN3 is also applicable to genome edited plants. This position is in line with the conclusions of the opinion stating that the EFSA guidances- are sufficient but can be only partially applied for the risk assessment of plants generated by the application of SDN-1, SDN-2, and ODM methods, especially when a transgene is not present in the final product. Moreover, the GMO Panel was not mandated to provide a comprehensive list of the studies required or not for the risk assessment of genome edited plants.	
ENSSER	3.2.2.2 Assessment of Section 4.2: Alteration to the genome - no text	General comment: This section should first establish (or reiterate in case it is to be covered earlier in the final opinion) which kind of alterations to the genome may occur for SDN-1 and SDN-2, also including the processes applied, such as modifications derived through Agrobacterium, gene gun, protoplast transformation etc, • intended/unintended sequence modifications • at or near the target site (on-target site) as well as elsewhere in the genome (sometimes described as "off-target" sites, or "off target" effects of the SDN) • epigenetic alterations the term 'insertion site' is wrong for SDN-1 Furthermore: Whilst having the same cutting /cleavage mechanisms, the ensuing repair mechanisms are vastly different for SDN-1 and SDN-3, thus not directly comparable or directly expected to result in the same outcomes. We reccomended to consider a differently structured section of "Alteration to the genome" for SDN-1 and SDN-2 (as compared to the SDN-3 opinion)	The GMO Panel takes note of the comment. The GMO Panel has developed this opinion by strictly adhering to the terms of reference mandating EFSA to provide opinion on very specific questions. Accordingly, the section 3.2.2. closely follows the section four of the SDN-3 opinion. This opinion is not intended to replace SDN-3 opinion or to provide a comprehensive update on SDN technology; however, a brief update on technology development is included, including update on on-target and off-target sites in genomes. Moreover, the EFSA GMO panel has concentrated on differences in SDN-1, SDN-2 and ODM technologies compared to SDN-3, while the general processes, such as delivering genome editing reagents in the cell, which are common to SDN-1,	189

			SDN-2 and SDN-3 were considered less relevant. The text of the relevant sections in the opinion has been updated.	
Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG)	3.2.2.2 Assessment of Section 4.2: Alteration to the genome - no text	line 318: add after transgene - intragene or cisgene line 319: add after specific genes - or alleles	The GMO Panel takes note of the comment. The text has been modified to better reflect the content of the EFSA opinion on SDN-3.	190
GMO Office, National Institute of Public Health and the Environment (RIVM)	3.2.2.2 Assessment of Section 4.2: Alteration to the genome - no text	Lines 343-345, 352-354 We agree with the statement on line 343-345 …' backcrossing following the transformation process will remove these potential off-targets from the final product, except for those that are genetically linked to the intentionally modified locus (Hahn and Nekrasov, 2019)' and the statement on line 352-354 ….. 'because off-target effects in SDN- and ODM-based approaches is negligible compared to conventional plant breeding, the GMO Panel considers that the analysis of potential off-targets would be of very limited value for the risk analysis'. However, in line 359 (and further) EFSA mentions that unintentional insertion of fragments of 'any exogenous DNA deployed during the process' can occur and that therefore 'the 'applicant should demonstrate that the genome of the end product is free from any DNA sequence potentially derived from the methods used to generate the SDN-type of modification (e.g. plasmids or vectors, section 3.1.3)'. It would be good to state in the opinion that, as is mentioned for off-target effects, also potential fragments of exogenous DNA (if any) will probably be removed by backcrossing following the transformation process, except for those that are genetically linked to the intentionally modified locus.	The GMO Panel takes note of the comment. The text of the relevant sections in the opinion has been updated.	191
National Food Institute, Technical University of Denmark	3.2.2.2 Assessment of Section 4.2: Alteration to the genome - no text	A number of different possible hazards are mentioned in section 4.2 of the SDN-3 opinion as relevant for SDN-3. In this opinion these are not taken into consideration. A number of these hazards could be relevant using SDN-1/SDN-2 as well, like creation of novel ORFs depending of the size of the modification. However, the outcome would be similar to using conventional mutagenesis.	The GMO Panel agrees that the alterations to the genome at target site or at off-target site would be similar to those obtained using conventional mutagenesis, except that there would be fewer mutations. This opinion compares SDN-1, SDN-2 and ODM technologies to SDN-3 opinion, while the general risk assessment procedures to identify unintended effects, such as comparative assessment are still valid.	192
Plantum - Netherlands seed association	3.2.2.2 Assessment of Section 4.2: Alteration to the genome - no text	We are pleased to note that "SDN-1 and SDN-2 approaches "produce only a fraction, if any, of all the unintended genomic alterations introduced in conventional breeding"	The GMO Panel takes note of the comment.	193

Umweltbundesamt (Environment Agency Austria) on behalf of the Austrian lead Competent Authority, the Federal Ministry of Social Affairs, Health, Care and Consumer Protection.	3.2.2.2 Assessment of Section 4.2: Alteration to the genome - no text	Line 311ff: We refer to our comments to the previous sections concerning the necessary revision of this section. We note that some recent publications describe issues which need to be taken into account during risk assessment of SDN-1, SDN-2, and ODM approaches (Agapito-Tenfen et al., 2018; Eckerstorfer et al., 2019a; Zhao and Wolt, 2017). The revision should also address that information on the off-target mechanism and frequency for ODM is still limited (Modrzejewski et al., 2019). Therefore we suggest that a comprehensive stepwise assessment as outlined in Eckerstorfer et al. (2019a) is recommended. Line 322-324: The draft opinion concludes that the section 4.2.1 of the EFSA opinion on SDN-3 ("Alteration at the insertion site") is not applicable to plants developed by SDN-1, SDN-2, and ODM approaches. However this previous opinion mentions that SDN-3 transgenes may be integrated also via NHEJ pathways. "Insertion at a target locus by NHEJ can be accompanied by all of the types of genomic changes that have been reported for the NHEJ repair, including DNA deletions, insertions (including "filler" DNA, i.e. short sequences new to the plant), duplications and inversions. Such changes at the insertion site have been observed on many occasions in transgenic plants (EFSA, 2012)." We note that this conclusion is highly relevant to all SDN-1 und SDN-2 applications that create modifications by insertion of any sequence via the NHEJ mechanism. Lines 368-370: We agree with the conclusion that section 4.2.2 of the EFSA opinion on SDN3 ("Alteration elsewhere in the genome") is applicable to plants developed by SDN-1, SDN-2, and ODM approaches. However we suggest that a comprehensive stepwise assessment of unintended genetic and phenotypic changes as outlined in Eckerstorfer et al. (2019a) is recommended. Agapito-Tenfen, Sarah Z.; Okoli, Arinze S.; Bernstein, Michael J.; Wikmark, Odd-Gunnar; Myhr, Anne I. (2018): Revisiting Risk Governance of GM Plants: The Need to Consider New and Emerging Gene-Editing Techniques. In: Frontiers in plant science 9, S. 1874. DOI: 10.3389/fpls.2018.01874. Eckerstorfer, Michael F.; Dolezel, Marion; Heissenberger, Andreas; Miklau, Marianne; Reichenbecher, Wolfram; Steinbrecher, Ricarda A.; Waßmann, Friedrich (2019a): An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Tech-niques (nGMs). In: Frontiers in bioengineering and biotechnology 7, S. 31. DOI: 10.3389/fbioe.2019.00031. Modrzejewski, Dominik; Hartung, Frank; Sprink, Thorben; Krause, Dörthe; Kohl, Christian; Wilhelm, Ralf (2019): What is the available evidence for the range of applications of ge-nome-editing as a new tool for plant trait modifica-tion and the potential occurrence of associated off-target effects: a systemat-ic map. In: Environ Evid 8 (1). DOI: 10.1186/s13750-019-0171-5. Zhao, Hui; Wolt, Jeffrey D. (2017): Risk associated with off-target plant ge-nome editing and methods for its limitation. In: Emerging Topics in Life Sci-ences 1 (2), S. 231–240. DOI: 10.1042/ETLS20170037.	Regarding comment to line 311, to develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references. Regarding comment to lines 322-324, the EFSA GMO panel is aware of the possible alterations at the target site in SDN-1 and SDN-2 approaches. These alterations would be assessed as part of the risk assessment of SDN-1 and SDN-2 plants. However, in SDN-1 and SDN-2 no exogenous DNA is inserted in genomes, which justifies the conclusion on lines 322-324. Moreover, this opinion compares SDN-1, SDN-2 and ODM technologies to SDN-3 opinion, while the general risk assessment procedures to identify unintended effects, such as comparative assessment are still valid.	194
GenØk-centre for biosafety	3.2.2.2 Assessment of Section 4.2: Alteration to the genome - no text	For details regarding this section: please read our attached table with our comments. **Copied from the submitted pdf file:** Again: The presence of foreign DNA should not be the only criteria for analysis In lines 315-317, EFSA states "Irrespective of the approach used, the successful application of SDN-1, SDN-2, and ODM results in a sequence modification which is targeted to a specific predetermined	The opinion was prepared in response to a mandate to compare SDN-1, SDN-2 and ODM technologies to SDN-3 opinion. The major difference in SDN-1, SDN-2 and ODM scenarios compared to the SDN-3, is the	195

		genomic locus and no exogenous DNA is inserted." It further confirms that for these reasons, the investigation of several aspects of the insertion site are not relevant for plants developed thought these techniques. EFSA again limited its analysis to the presence or absence or exogenous DNA, omitting the need to verify integration events as well as other aspects of genetic modification. For example, CRISPR is widely used to disrupt gene function by inducing small insertions and deletions like those present in SDN-1 and SDN-2 approaches. There has been evidence that some single-guide RNAs (sgRNAs) can induce small insertions or deletions that partially alter splicing or unexpected larger deletions that remove exons6. Exon skipping adds to the unexpected outcomes that must be accounted for in RA.	absence of insertion of foreign DNA at the target site. However, the target site for genome editing still needs to be characterized as part of the molecular characterization. Moreover, if exogenous DNA is still present elsewhere in the genome, e.g., as a CRISPR-Cas9 construct, such plants will be considered as conventional GMOs, and will require appropriate characterization. Unintended changes at off-target sites in the genome will be assessed as part of comparative assessment. The text of the opinion has been updated accordingly.	
CropLife Canada	3.2.2.2 Assessment of Section 4.2: Alteration to the genome - no text	Lines 352 – 354: CropLife Canada agrees that providing the analysis of potential off-target effects is of limited value for plants developed using SDN and ODM approaches given their targeted nature. In the final report, it would be of benefit for concerned stakeholders if EFSA could identify here a list of example studies that are currently part of the guidance which are solely focused on off-target analysis and that do not apply to these technologies. For instance, general untargeted comparative compositional and agronomic analysis often used to confirm the lack of unintended effects should not apply to SDN-1, SDN-2 and ODM given that their targeted nature produce changes similar to what can occur in conventional breeding, which has been used in thousands of varieties with no cases of toxins or allergens introduced.	The GMO Panel takes note of the comment. The GMO Panel has developed this opinion by strictly adhering to the terms of reference mandating EFSA to provide opinion on very specific questions. Development of new guidance document for risk assessment of genome edited plants was not required, since the Commission IR No 503/2013 is still broadly applicable. Depending on the method which was used to generate the genome edited plant and the traits characterizing such products, the GMO panel may consider some data requirements not necessary for the risk assessment. For this reason, the "case-by-case" approach as described in the opinion on SDN-3 is also applicable to genome edited plants. This position is in line with the conclusions of the opinion stating that the EFSA guidances are sufficient but can be only partially applied for the risk assessment of	196

			plants generated by the application of SDN-1, SDN-2, and ODM methods, especially when a transgene and/or exogenous DNA is not present in the final product.	
ENSSER	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	"insertion site" is the wrong term for SDN-1, this should be stated here. L311: Add following new sentence after the first: "Contrary to SDN-3, SDN-1 evokes a different repair mechanism called NHEJ, which follows different rules, and occurs at a different time of the cell cycle than HDR, which is the repair mechanism necessary for SDN-3 (and SDN-2) to work." This may have different risk implications and needs thus to be highlighted here. See also point to "fewer" line 341. L313-315: These are new and distinctly different, in particular prime editing, and hold their own risks. They should not be considered as SDN-2, as they do not follow the mechanisms, instead, they should have an own section or assessment, although for prime editing there will be very little data as yet. Please remove the sentence and create a new section with recommendation for own assessment. L315: Why "the"? A successful application? Please include the following in the sentence after ODM: "ideally and if performing according to design (as intended) …. " L320-321: largely correct, if working as intended. Though insertion may happen never the less (as also pointed out for SDN-3, that NHEY that and me L322: It is correct that there is no intended "insertion" (at least not for SDN-1, as definition of SDN-2 still not clear) and thus no insertion site as such and thus the section 4.2.1.. But there is a "target site" – so where is the assessment component for this??? Problems with EFSA 2012 SDN-3 opinion – section 4.2.1: è many claims and statement, yet no literature! è When will SDN-3 occur via NHEJ and when via HDR – opinion seems to suggest that HR will automatically take place and no unexpected alterations will occur at insertion site if design of donor DNA is accordingly, yet plenty of alterations may occur with NHEJ insertions. Where is the comparison please between NHEJ insertion of the same construct, and HR, and comparison of with or without SDN.	The GMO Panel agrees that the concept of insertion site is not applicable for the target site for the genetic modification using SDN- and ODM-based approaches. The title of the section refers to the corresponding section of the SDN-3 opinion that is assessed for its applicability to the risk assessment of plants developed using SDN- and ODM-based approaches. Indeed, the GMO Panel concludes that the section 4.2.1 of the EFSA opinion on SDN-3 ("Alteration at the insertion site") is not applicable to plants developed by SDN-1, SDN-2, and ODM approaches. Regarding comment for line 311, the GMO Panel refers to the mechanism to induce DSB rather than the repairing mechanisms. Regarding comments for line 313-315, 320-321, and 322, the GMO Panel consider the text sufficiently clear. Moreover, the GMO Panel was mandated to assess the applicability of the different sections of the opinion on SDN-3 to plant generate via SDN-1, SDN-2, and ODM approaches and not to provide new guidances on the risk assessment of these products.	197
EuropaBio	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	Lines 322-324: EuropaBio agrees with the conclusion of the EFSA GMO Panel in this section. As there is no insertion, this section is not applicable. EuropaBio considers that EFSA should clarify here that applying the problem formulation approach, this could be easily established and a simple justification	The GMO Panel concludes that the section 4.2.1 of the EFSA opinion on SDN-3 ("Alteration at the insertion site") is not applicable to plants developed by SDN-1, SDN-	198

		can be provided to address this section without the need to generate specific data or revise existing EFSA guidance.	2, and ODM approaches. Moreover, the GMO Panel concludes that the existing Guidances for food and feed (EFSA GMO Panel, 2011) and environmental risk assessment (EFSA GMO Panel, 2010) are sufficient but can be only partially applied for the risk assessment of plants generated via SDN 1, SDN 2, and ODM approaches. Therefore, the document does not propose that specific data are required other than those included in the EFSA guidance.	
Logos Environmental	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	Although "DNA-free" SDN is possible, most genome-edited plants developed to date utilise insertion of a cassette containing DNA coding for the genome editing components. This insertion can cause fragments and rearrangements, independent of any genomic irregularities caused by the genome editing process. This unintended effect needs to be considered as part of the risk assessment for genome-edited GMO crops, as it is with first generation GMOs. In addition, the use of genetic insertion followed by out breeding, raises the question of how a suitable comparator can be found, as any errors arising from the insertion will still be present. There is a whole swath of possible errors caused by genome editing that are absolutely vital to the risk assessment, yet have been omitted by EFSA. Some of these errors may, as yet, only been reported (or predominantly reported) in genome-edited animals, but the same considerations apply to genome-edited plants. For example, whether the altered gene has more than one function and whether the change might have altered this additional function (Burkard et al. (2017) PLoS Pathog. 13: e1006206). Of crucial importance is the possibility of "exon skipping" (Kapahnke et al. (2016) Cells 5: 4 5. Lalonde (2017) PLoS One 12: e0178700; Mou et al. (2017) Genome Biol. 18: 108; Tuladhar (2019) Nat. Commun. 10 (1):4056. doi:10.1038/s41467-019-12028-5; Hahn & Nekrasov (2019) Plant Cell Rep. 38: 437-441.) Such alterations could lead to changes that could be important in terms of environmental and food/feed safety as they could create aberrant proteins.	With respect to the unintended insertion of DNA fragments from the insertion cassette, in section 3.2.2.2.2., the document proposes that "If the final product is not intended to retain any exogenous DNA, the applicant should assess the potential presence of a DNA sequence derived from the methods used to generate the SDN modification (e.g. plasmids or vectors, see section 3.1.3).". This is also in line with the current risk assessment of GMO performed under EU regulation. The GMO Panel considers that genome alterations produced by SDN- and ODM-based approaches are of the same type as those produced by natural variation and by the application of several techniques used in conventional breeding. The product is risk assessed 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.	199

Association Française de Biotechnologies Végétales	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	AFBV edits and comment: Line 318: Consistently with Lines 156, 236 and 293, insert after "transgene" the words "an intragene or a cisgene". Line 319: insert "or alleles" after the word "genes". When using SDN-3 to insert a cisgene, one can insert a new cisgene present in the donor plant and absent from the receiving plant or a new allele for a gene already present in the receiving plant.	Regarding the comment for line 318, the expression "DNA sequence" were inserted in line 236 and 293 but not in line 156 because the text in that section is derived from the opinion on SDN-3. The expression "or alleles" has not been inserted in line 319 because this is the wording of the EFSA opinion on SDN-3.	200
Julius Kühn-Institut	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	L317: Replace "and" by "where" .	The text has been amended accordingly.	201
European Coordination Via Campesina	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	Most genome-edited plants developed to date utilise insertion of a cassette containing DNA coding for the genome editing components. This insertion can cause fragments and rearrangements, independent of any genomic irregularities caused by the genome editing process. This unintended effect needs to be considered as part of the risk assessment for genome-edited GMO crops, as it is with first generation GMOs. In addition, the use of genetic insertion followed by out breeding, raises the question of how a suitable comparator can be found, as any errors arising from the insertion will still be present. There is a whole swath of possible errors caused by genome editing that are absolutely vital to the risk assessment, yet have been omitted by EFSA. Some of these errors may, as yet, only been reported (or predominantly reported) in genome-edited animals, but the same considerations apply to genome-edited plants. For example, whether the altered gene has more than one function and whether the change might have altered this additional function (Burkard et al. (2017) PLoS Pathog. 13: e1006206). Of crucial importance is the possibility of "exon skipping" (Kapahnke et al. (2016) Cells 5: 4 5. Lalonde (2017) PLoS One 12: e0178700; Mou et al. (2017) Genome Biol. 18: 108; Tuladhar (2019) Nat. Commun. 10 (1):4056. doi:10.1038/s41467-019-12028-5; Hahn & Nekrasov (2019) Plant Cell Rep. 38: 437-441.) Such alterations could lead to changes that could be important in terms of environmental and food/feed safety as they could create aberrant proteins.	With respect to the unintended insertion of DNA fragments from the insertion cassette, in section 3.2.2.2.2., the document proposes that "If the final product is not intended to retain any exogenous DNA, the applicant should assess the potential presence of a DNA sequence derived from the methods used to generate the SDN modification (e.g. plasmids or vectors, see section 3.1.3).". This is also in line with the current risk assessment of GMO performed under EU regulation. The GMO Panel considers that genome alterations produced by SDN- and ODM-based approaches are of the same type as those produced by natural variation and by the application of several techniques used in conventional breeding. The product is risk assessed for possible unintended effects by the assessment of studies including phenotypic and the compositional analysis of the	202

			GM plant, as laid down on IR 503/2013. and EFSA guidances.	
French agency for Food, Environmental and Occupational Health & Safety (Anses)	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	Page 9, line 317: Proposal to replace "For these reasons, several considerations described..." by "For these reasons, the considerations described...", because all the considerations described in section 4.2.1 of the EFSA opinion on SDN-3 are concerned (consistency with lines 322-324).	The text has been amended accordingly.	203
European Plant Science Organisation, EPSO	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	Line 322 to 324: EPSO agrees with the conclusion by the GMO panel that the opinion on SDN-3 ("Alteration at the insertion site", section 4.2.1) does not apply to plants developed by SDN-1, SDN-2, and ODM approaches.	The GMO Panel thanks for the comment.	204
Nature et Progrès Belgique	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	Although "DNA-free" SDN is possible, most genome-edited plants developed to date utilise insertion of a cassette containing DNA coding for the genome editing components. This insertion can cause fragments and réarrangements, independent of any genomic irregularities cause by the genome editing process. This unintended effect needs to be considered as part of the risk assessment for genome-edited GMO crops, as it is with first generation GMOs. In addition, the use of genetic insertion followed by out breeding, raises the question of how a suitable comparator can be found, as any errors arising from the insertion will still be present. There is a whole swath of possible errors caused by genome editing that are absolutely vital to the risk assessment, yet have been ommitted by EFSA. Some of these errors may, as yet, only been reported (or predominantly reported) in genome-edited animals, but the same considerations apply to genome edited plants. For example, whether the altered gene has more than one function and whether the change might have altered this additional function (Burkard et al. (2017) PLos Pathol. 13:e1006206). Of crucial importance is the possibility of "exon skipping" (Kapahnke et al (2016) Cells 5: 4 5; Lalonde (2017) PLos One 12 e0178700; Mou et al (2017) Genome Biol. 18:108; Tuladhar (2019) Nat. Commun. 10 (1) :4056 doi:10.1038/s41467-019-12028-5; Hahn & Nekrasov (2019) Plant Cell Rep. 38:437-441). Such alterations could lead to changes that could be important in terms of environmental and food/feed safety as they could create aberrant proteins.	With respect to the unintended insertion of DNA fragments from the insertion cassette, in section 3.2.2.2.2., the document proposes that "If the final product is not intended to retain any exogenous DNA, the applicant should assess the potential presence of a DNA sequence derived from the methods used to generate the SDN modification (e.g. plasmids or vectors, see section 3.1.3).". This is also in line with the current risk assessment of GMO performed under EU regulation. The GMO Panel considers that genome alterations produced by SDN- and ODM-based approaches are of the same type as those produced by natural variation and by the application of several techniques used in conventional breeding. The product is risk assessed 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.	205

Haut Conseil des biotechnologies (High Council for Biotechnology)	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	l.311-313. Suggestion to replace "On the contrary" by "Unlike the SDN-1 and SDN-2 approaches" l. 317. "and no exogenous DNA is inserted". Is it documented that it is always the case for SDN-2? (See interrogations in 3.1.1).	Regarding the comment for lines 311-313, the text has been amended accordingly. Regarding comment to line 317, the GMO Panel considers the sentence correct. For SDN-2, the homologous recombination mechanism that will copy the information carried by the donor template will necessarily involve a DNA synthesis step. Therefore, there is no *sensu stricto* integration of the donor template (=exogenous DNA).	206
Testbiotech	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	[Line 322 to 324, delete and replace text] "However, in regard alterations at the insertion site, all steps of the process have to be taken into account, such as the delivery of the SDN machinery by transgene insertion (see 3.1.3 and 3.1.4). Further, in regard to SDN-1, SDN-2 (and potentially also ODM), a broad range of unintended on-target effects have to be considered. There are several examples of specific unintended on-target-effects described in existing publications: for example, a general problem with DNA-based CRISPR/Cas9 is the unintended insertion of the DNA or partial DNA-fragments encoding the CRISPR/Cas9 complex itself into the genome of the plant (see Liang et al., 2017). Further, large deletions and complex rearrangements have been reported during the CRISPR/Cas9 process. This has been shown to be the case particularly in human and animal cells (Kosicki et al., 2018). According to Hahn and Nekrasov (2019), such effects can very likely also occur in plants, but the methodologies to identify these effects are hardly ever used. Thus, such on-target effects might often be overlooked in plants. In addition, large deletions induced by a single guide RNA were found to delete whole exons causing exon skipping in cell lines (Mou et al., 2017; Sharpe and Cooper, 2017; Kapahnke et al., 2016; Tuladhar et al., 2019). Exon skipping can produce mRNAs with intact reading frames that encode altered, partially functional proteins which have to be assessed in risk assessment. It is essential to apply available methods carefully to analyze the genome in order to detect such unintended effects, and that the specific methods applied during the genetic engineering of an organism are known and taken into account during risk assessment. In some cases the data set needed to perform risk assessment of SDN-1, SDN-2 (and potentially also ODM) might go far beyond those needed for SDN-3, e.g. if many copies of one genes are altered or several genes are targeted by multiplexing (see 3.1.2). In regard to environmental risks, these unintended on-target effects can also cause changes in the composition of plants that may impact the food web, changes in the composition of plants which may influence plant communication and interaction with the environment, changes in the fitness and next generation effects of plants with the potential to persist and propagate in the environment (for overview see Testbiotech, 2020; see also Bauer-Panskus et al., 2020).	To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references.	207

		There are several publications showing a broad range of aspects that have to be taken into account in regard to the safety of on-target effects in organisms derived from SDN-1 and SDN-2 processes (for overview, Agapito-Tenfen et al., 2018, Eckerstorfer et al., 2019; Testbiotech, 2020, Cotter et al., 2020; Kawall et al., 2020). While an increasing number of publications have investigated unintended on-target effects for SDN-based technologies, information on the off-target mechanism and frequency for ODM is limited (Modrzejewski et al., 2019). Due to the lack of information, the panel found it difficult to reach to any conclusions. Due to all the above considerations, the GMO panel concludes that the section 4.2.1 of the EFSA opinion on SDN-3 ("Alteration at the insertion site") is partially applicable, but in many cases will not be sufficient to assess the risks of plants developed by SDN-1, SDN-2, and ODM applications.		
Sciensano	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	Line 311: "SDN-1 and SDN-2 approaches use the same molecular mechanisms to induce DSBs as the SDN-3" => this is only valid if approaches such as base editing and prime editing are not considered as the latter do not induce DSB. This is however rectified at line 314.	The GMO Panel thanks for the comment.	208
Federal Agency for Nature Conservation	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	Lines 311-324: Further unintended changes at or near the target site have been described for genome editing including large deletions or inversion (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). Therefore, the potential DNA-repair outcomes resulting from individual CRISPR-induced DSBs may be significantly larger and more complex than previously anticipated (Thomas et al. 2019; also Kapahnke et al. 2016). Although mainly detected in animal and human cells these unintended on-target damages should be considered for SDN-1, SDN-2 and ODM interventions in plants as well, because (i) some of them have been reported for plants as well (Hahn and Nekrasov 2019) and (ii) the proper technique has rarely been applied yet (Mou et al. 2017, Hahn and Nekrasov 2019). Besides, these kinds of unintended on-target changes might occur at off-target sites as well! Hahn, Florian; Nekrasov, Vladimir (2019): CRISPR/Cas precision: do we need to worry about off-targeting in plants? In: Plant Cell Rep 38 (4), p. 437–441. DOI: 10.1007/s00299-018-2355-9. Kapahnke, Marcel; Banning, Antje; Tikkanen, Ritva (2016): Random Splicing of Several Exons Caused by a Single Base Change in the Target Exon of CRISPR/Cas9 Mediated Gene Knockout. In: Cells 5 (4). DOI: 10.3390/cells5040045. Kosicki, M., Tomberg, K. & Bradley, A. Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. Nat. Biotechnol. https://doi.org/10.1038/nbt.4192 (2018). Mou, Haiwei; Smith, Jordan L.; Peng, Lingtao; Yin, Hao; Moore, Jill; Zhang, Xiao-Ou et al. (2017): CRISPR/Cas9-mediated genome editing induces exon skipping by alternative splicing or exon deletion. In: Genome biology 18 (1), p. 108. DOI: 10.1186/s13059-017-1237-8.	SDN-1 and SDN-2 approaches are defined by the strategy but also the outcome of the modification at the target site. Plants containing on-target alterations such as large unintended deletions or rearrangement should not be considered as SDN-1 or SDN-2 edited plants. In any case, the GMO Panel reminds that the characterization of the alterations at the target site, 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- and ODM-based methods.	209

		Sharpe, Joshua J.; Cooper, Thomas A. (2017): Unexpected consequences. Exon skipping caused by CRISPR-generated mutations. In: Genome biology 18 (1), p. 109. DOI: 10.1186/s13059-017-1240-0.		
Envirnonmental association Za Zemiata	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	[Line 322 to 324, delete and replace text] "However, in regard alterations at the insertion site, all steps of the process have to be taken into account, such as the delivery of the SDN machinery by transgene insertion (see 3.1.3 and 3.1.4). Further, in regard to SDN-1, SDN-2 (and potentially also ODM), a broad range of unintended on-target effects have to be considered. There are several examples of specific unintended on-target-effects described in existing publications: for example, a general problem with DNA-based CRISPR/Cas9 is the unintended insertion of the DNA or partial DNA-fragments encoding the CRISPR/Cas9 complex itself into the genome of the plant (see Liang et al., 2017). Further, large deletions and complex rearrangements have been reported during the CRISPR/Cas9 process. This has been shown to be the case particularly in human and animal cells (Kosicki et al., 2018). According to Hahn and Nekrasov (2019), such effects can very likely also occur in plants, but the methodologies to identify these effects are hardly ever used. Thus, such on-target effects might often be overlooked in plants. In addition, large deletions induced by a single guide RNA were found to delete whole exons causing exon skipping in cell lines (Mou et al., 2017; Sharpe and Cooper, 2017; Kapahnke et al., 2016; Tuladhar et al., 2019). Exon skipping can produce mRNAs with intact reading frames that encode altered, partially functional proteins which have to be assessed in risk assessment. It is essential to apply available methods carefully to analyze the genome in order to detect such unintended effects, and that the specific methods applied during the genetic engineering of an organism are known and taken into account during risk assessment. In some cases the data set needed to perform risk assessment of SDN-1, SDN-2 (and potentially also ODM) might go far beyond those needed for SDN-3, e.g. if many copies of one genes are altered or several genes are targeted by multiplexing (see 3.1.2). In regard to environmental risks, these unintended on-target effects can also cause changes in the composition of plants that may impact the food web, changes in the composition of plants which may influence plant communication and interaction with the environment, changes in the fitness and next generation effects of plants with the potential to persist and propagate in the environment (for overview see Testbiotech, 2020; see also Bauer-Panskus et al., 2020). There are several publications showing a broad range of aspects that have to be taken into account in regard to the safety of on-target effects in organisms derived from SDN-1 and SDN-2 processes (for overview, Agapito-Tenfen et al., 2018, Eckerstorfer et al., 2019; Testbiotech, 2020, Cotter et al., 2020). While an increasing number of publications have investigated unintended on-target effects for SDN-based technologies, information on the off-target mechanism and frequency for ODM is limited (Modrzejewski et al., 2019). Due to the lack of information, the panel found it difficult to reach to any conclusions. Due to all the above considerations, the GMO panel concludes that the section 4.2.1 of the EFSA	To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references.	210

		opinion on SDN-3 ("Alteration at the insertion site") is partially applicable, but in many cases will not be sufficient to assess the risks of plants developed by SDN-1, SDN-2, and ODM applications.		
Corporate Europe Observatory	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	If the insertion of a cassette containing DNA coding for the genome editing components is utilised, this insertion can cause fragments and rearrangements. This unintended effect needs to be considered as part of the risk assessment for genome-edited GMO crops, as it is with first generation GMOs. The risk assessment should take into account possible errors caused by genome editing but that are not taken into account in this Opinion by EFSA. Some examples: - whether the altered gene has more than one function and whether the change might have altered this additional function (Burkard et al. (2017) PLoS Pathog. 13: e1006206). - the possibility of "exon skipping" (Kapahnke et al. (2016) Cells 5: 4 5. Lalonde (2017) PLoS One 12: e0178700; Mou et al. (2017) Genome Biol. 18: 108; Tuladhar (2019) Nat. Commun. 10 (1):4056. doi:10.1038/s41467-019-12028-5; Hahn & Nekrasov (2019) Plant Cell Rep. 38: 437-441.) These alterations can have impacts on food/feed safety.	With respect to the unintended insertion of DNA fragments from the insertion cassette, in section 3.2.2.2.2., the document proposes that "If the final product is not intended to retain any exogenous DNA, the applicant should assess the potential presence of a DNA sequence derived from the methods used to generate the SDN modification (e.g. plasmids or vectors, see section 3.1.3).". This is also in line with the current risk assessment of GMO performed under EU regulation. Moreover, the GMO Panel reminds that the characterization of the alterations at the target site, 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- and ODM-based methods.	211
BUND e.V. / Friends of the Earth Germany	3.2.2.2.1 Alteration at the insertion site [Section 4.2.1]	Line 316 ADD between "to a .. specific": "more or less" Line 324 ADD new sentence after "approaches": "Still, there is strong evidence in the literature that on-target effects at the target/insertion site are common, e.g. genomic and foreign DNA may be inserted at the target site (Andersson et al. 2018). But even without the insertion of foreign DNA, on-target effects can lead, among others, to new mRNA populations, exon skipping and sequence rearrangements (Kapahnke et al. 2016, Lalonde et al. 2017, Mou et al. 2017, Smits et al. 2019, Sharpe & Cooper 2017, Tuladhar et al. 2019, Kosicki et al. 2018). These potential effects have to be analyzed and assessed, in order to prevent negative consequences for the environment and human health." Additional comment: The opinion in its present form does not take into consideration that the insertion itself can cause fragments and rearrangements, independent of any genomic irregularities caused by the genome editing process. This unintended effect needs to be considered as part of the risk assessment for genome-edited GMO crops, as it is with first generation GMOs.	Regarding comment for line 316, the GMO Panel considers that the text is sufficiently clear. Regarding the comment for line 324, SDN-1 and SDN-2 approaches are defined by the strategy but also by the outcome of the modification at the target site. Plants containing on-target alterations such as large unintended deletions or rearrangement should not be considered as SDN-1 or SDN-2 edited plants. In any case, the GMO Panel reminds that the characterization of the alterations at the target site, which is part of	212

		New findings in literature show that genome editing may possibly cause errors that must be part of the risk assessment, e.g. exon skipping and its effects. Research related to those questions must become part of this opinion.	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- and ODM-based methods. With respect to the unintended insertion of DNA fragments from the insertion cassette, in section 3.2.2.2.2., the document proposes that "If the final product is not intended to retain any exogenous DNA, the applicant should assess the potential presence of a DNA sequence derived from the methods used to generate the SDN modification (e.g. plasmids or vectors, see section 3.1.3)". This is also in line with the current risk assessment of GMO performed under EU regulation.	
ENSSER	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	General and overarching points: This section requires an introductory sentence. L326: Please insert/add the following text as a first new paragraph: Alterations elsewhere in the genome – i.e. other than at or near the target site - occur either (a) due to so-called "off-target activity" of the SDNs (…to a lower efficiency degree) or (b) due to the application of these techniques and approaches, including the delivery of the SDNs either as DNA constructs and other plant transformation or transfection processes, the delivery of mRNA, proteins or ribonucleoproteins, protoplast technology, specific in-vitro cell and tissue culture technologies and processes, etc., which is often included in the term "off-target effects" or (c) due to the presence of 'exogenous' nucleic acids, including for example vector sequences, expression constructs, single-stranded or double-stranded nucleic acids present in the culture or application medium or spray, etc. which may for example result in unintended small or large insertions, or epigenomic changes (Eckerstorfer 2019). Combined, all these modifications can be referred to as "application-based process-induced genome-wide modifications" or "application-based process-induced genome-wide effects". L326-27: The phrasing chosen in line 326 is not correct. SDN-1 do not result in the "precise modification" nor do they necessarily result in the "intended modification" at a predetermined genomic sequence. Rather the mutation/modification is and will be unpredictable in itself, i.e. in its sequence alteration, due to the error-prone NHEJ repair pathway, as also outlined in this opinion. In fact - contrary to 'precise modification' - this draft opinion has referred to the same type of mutation as "random	The GMO Panel takes not of this comment. This opinion, and the scientific literature and the opinions of the European Commission SAM on NBTs cited, appropriately describe the specificity and precision of the SDN-1, SDN-2 and ODM approaches. Regarding the comment for lines 326-327, the text has been revised to indicate that overall SDN-1, SDN-2, and ODM aim at modifying a predetermined plant genomic sequence(s). Regarding comment to line 328, the text has been revised. The GMO Panel was not mandated to provide neither a	213

		mutation" previously (line 197) , which also does not seem the right terminology in the given context. Furthermore, SDNs are not "precise" as such, but rather have a high efficiency in setting DSBs at predetermined sites, albeit also having the capacity, to a much lower efficiency, to set DSBs at other sites, as detailed in lines 327-329. Unintended on-target effects have also been observed with SDN-s (Hajiahmadi et al. 2019), thus the statement is not correct. As the main point is the lead-up to off-target alterations, we suggest correcting the sentence by moving 'in general' and deleting 'precise and intended' and thus to read: "The application of SDN-1, SDN-2, and ODM approaches result [in general] in the modification of predetermined plant genomic sequence(s)." L328: For clarity and accuracy, please insert "and off-target effects" and "and their processes" for the sentence to read: " ……. because of the off-target activity and off-target effects associated with these applications and their processes." L329: Please define and elaborate what is meant by "the specificity of the technology used". As reported in the scientific literature, target efficiency, including on-target and off-target activities, depend on a whole set of parameters and variables, experimental parameters such as: (i) the specific nuclease(s) used; (ii) the target organism and its tissue, respectively; (iii) the targeted gene(s); (iv) the way in which the components are introduced into the cells; (v) the dosage of the nuclease(s); (vi) which CRISPR/Cas, the guide RNA used and (vii) duration of the intervention. With increasing experimentation and improvement of conditions, concentrations, modified cas molecules, diff PAM requirements etc. efficiency has been improving, and off-target effects appear to have gone down, yet research is still looking out for specific pre-determined or predefined off-target sites (Modrzejewski et al. (2019)), instead of looking in an unbiased manner, and utilising sequencing – such as long read next generation sequencing - and analytical methodologies that can detect both small mutations (small indels) as well as large mutations incl. translocations, inversions, deletions and insertions. Line 334: "that can either be predictable (for SDN-1 and SDN-2) …". Are thought to be predictable, yet see above and also see Akcakaya et al. 2018, who has been looking at algorithms and their limitations. L339: please add at the end, after 'reduce off-target effects': ", as well as the development (and/or identification) of other CRISPR-associated nucleases, such as Cas12 or Cpf1 …… ….. to help with efficiency and specificity and reduction in off-target effects. …." L340-42: The EFSA 2012 opinion quoted here - and in the role of being the central 'source' document for the SDN-1,-2, and ODM assessment and opinion - has a number of problems, other than being from the pre-CRISPR/Cas era and, understandably, out of date in its science. L341: Regarding "conventional mutagenesis": We find the choice of 'conventional mutagenesis' as a baseline and comparator problematic and and an avoidance to assess SDN-3 on its own terms. Mutagenesis – any type of mutagenesis – is as per definition of the 2001 Directive a GMO. Whilst mutagenesis that prior to 2001 already had a record and history of safe use is excempted from the obligations under the Directive	comprehensive literature review nor an horizon scan on the SDN-based technology. For this reason, the GMO Panel considers not to be necessary to include in the opinion the sections proposed in the comment regarding the variables and parameters which affect the target efficiency of these technologies. Regarding comment for line 334, the GMO Panel thanks for the comment. Regarding comment for line 339, the text has been improved by referring to the development of other CRISPR-associated nucleases. Regarding comment for lines 340-342, the GMO Panel thanks for the comment Regarding comment for line 341, 343, 345-347, 352-354, 354-358, the GMO Panel thanks for the comment. To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some	

		L341: Concerning the use of "fewer": EFSA 2012 does not provide any empirical evidence and data on quantity or quality of mutations in conventional breeding, in so-called "conventional" mutagenesis (see above L341) nor for SDN-3 applications (as performed by 2012). Neither does the current draft opinion provide and data or references of empirical studies, and in particular comparative studies, neither for SDN-3 applications, nor for SDN-1 or -2. The question of "fewer" or more, or whether these are the same type of mutations or not, or whether they occur at the same places or not, will depend on the methods and processes chosen, including which type of chemical, which type of radiation, which particular SDN combined with which gRNA (if CRISPR) and which particular delivery system (incl. in-vitro and cell/tissue culture conditions) and at which stage of cell cycle or plant development, for which length and at which concentration. Until full studies have been carried out, also including and accounting for the mutations arising from the accompanying and necessary processes, no such quantitative or qualitative assertion should be made. Indeed, it is not necessary if one treats each form in its own right and assesses them for hazards, risks and safety on their own terms. L343: "backcrossing following the transformation process will remove these potential off-targets…" Firstly, backcrossing will only remove all the unintended mutations present in the plant genome to the extent that backcrossing is being carried out, and where it is checked and tested. This cannot be taken as a given. Certainly not for genome editing, where a false sense of predictability is being pre-assumed, unless prolonged backcrossing, eg for 10 generations, becomes mandatory, including initial testing to ascertain the validity of this approach testing. L345-347: Without EFSA 2012 providing the relevant data re SDN-3, and without the EFSA 2020 draft providing the relevant data for the three technologies, assumptions of validity of the SDN-3 opinion for the current techniques are being made. The draft opinion states that "SDN-1 and SDN-2 approaches produce only a fraction, if any, of all the unintended genomic alterations introduced by conventional breeding." It declares so without supplying any data, in particular data of empirical and systematic parallel studies, and of studies where the baseline is conventional breeding in the traditional sense, and not automatically a product of mutational processes – which in themselves give rise to genetically modified organisms. It also does so despite comparing unintended Off-target effects mediated by genome editing with intended genetic variants that are generated by chemical and physical mutagenesis – which is problematic in itself. Before any statements about "fewer" or producing "only a fraction if any" of all the "unintended genomic alterations" as compared to "conventional breeding", firstly termes need to be defined, and categories clarified. Then the whole spectrum of conventional breeding techniques & approaches needs to be looked at, as well as all the SDN and ODM techniques and approaches. SDN-1 will give rise to different degree and extent of so-called off-target mutations depending not only on the sequences present, but also due to the protocols and conditions chosen, the plant chosen, the developmental stage, the stage of cell cycle when the nuclease is being active, the length and sequence and amount of guide RNA, the particular nuclease employed, the time span of when eg CRISPR/Cas is present and in which concentration. There will be settings, when the level of background mutations is high, as there will be settings when they are low.	additional relevant references (section 3.2.2.2.2). Regarding comment to line 362, with respect to the unintended insertion of DNA fragments from the insertion cassette, in section 3.2.2.2.2., the document proposes that "If the final product is not intended to retain any exogenous DNA, the applicant should assess the potential presence of a DNA sequence derived from the methods used to generate the SDN modification (e.g. plasmids or vectors, see section 3.1.3)". This is also in line with the current risk assessment of GMO performed under EU regulation.	

		The same will be true for chemically or physically (radiation) induced mutations. Depending on protocols and conditions, levels of mutations will be high, or they will be low. Depending on the purpose and the procedures and selection and breeding cycles that are to follow, high or low settings can be chosen. Furthermore, the product of SDN-1 application processes will contain numerous mutations derived from the various processess necessary to achieve the envisaged SDN-1 action in the plant. These mutations -the SDN-1 application-based process-induced modifications- are part and parcel to the products resulting from the SDN-1 procedures. They cannot be discounted when counting mutations – if counting mutations becomes the objective, as is being suggested in the current draft opinion. L352-354 "Therefore, because off-target effects in SDN- and ODM-based approaches is negligible compared to conventional plant breeding, the GMO Panel considers that the analysis of potential off-targets would be of very limited value for the risk analysis." The question arises, regarding quality, loci and impact of the mutations found in non-mutagenic conventional breeding, minimal-mutagenic conventional breeding and other pre-2001 conventional breeding with long history of safe use – as compared to any SDN- and ODM applications, including all the procedures and processes involved. This is particular of importance as 'genome editing' is being seen as a short-cut in producing new traits and new varieties, and to assure that 'short-cuts' are not taken to the detriment of environmental safety or human and animal health. We question the validity of and do not agree with this statement, especially in the absence of peer reviewed, systematic, empirical data, the current choice of baseline & comparator, and the assumption that having less numbers of mutations will make a product safe. CRISPR-based SDN-1 is a new and still developing technology (technique) and does not have a long history of safe use, nor a solid body of evidence. This requires in our opinion for the time being a solid risk assessment based on all evidence, and should avoid limiting evidence due to amongst other assumptions, or interpretations of terms. L354-358: This sentence contains important information that should form the basis of considerations, and not be an after-thought once conclusions have been made. Please adjust and move the sentence up higher and complement it with information on the limitation of using current algorithms (in silico) as compared to actual test (in vivo) as predictions for off-target effects/mutations (Akcakaya et al. 2018) L340-358: >>> we suggest urgently to delete the whole paragraph and to develop a new draft that is based on solid empirical data and the other considerations outlined above and that will distinguish between the different forms of breeding and not equate conventional breeding with mutational breeding, nor treat mutational breeding as one single entity nor SDN-1 and SDN-2. L359-367: This section covers aspect c) of the introductory paragraph (see General and overarching points, L326). L362: Not all sequences of exogenous DNA that may integrate into the recipient genome are necessarily known a priori. Whilst the insertion of vector DNA into DNA double-strand breaks is of concern, it is of perhaps even greater concern, that any other trace DNA present in the culture medium may be inserted into the host DNA. This at least has been shown to be of particular concern in animal		

		settings. Ono et al., 2019, for example identified the presence of goat DNA and bovine DNA in the genome of SDN-treated (i.e. genome edited) mice. This depended on whether goat or foetal calf serum had been used as a culture medium in the experiments. In fact, even retrotransposons had been transferred. In this context it becomes obvious that genome editing may unintentionally become a mechanism for horizontal gene transfer of not only foreign DNA but pathogens alike. Additionally to Norris et al. 2020 (mentioned in line 364), cases of unintended integration of non-host DNA include recipients such as mice (e.g. Ono et al., 2015, Ono et al. 2019 and Jeon et al. 2019) ; plants (e.g. Jacobs et al., 2015, Li et al., 2015); fish (Gutierrez-Triana et al., 2018).		
EuropaBio	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Lines 340-352: EuropaBio agrees that off-target changes induced by the application of SDN-1, SDN-2, SDN-3 and ODM are likely to be fewer than those occurring with conventional mutagenesis (also stated in lines 173-175) that have been used previously and have a long history of safe use or standing variation present in breeding populations. This is supported by the analysis conducted by the European Commission (New techniques in agricultural biotechnology. CEU. SAM_ADV, Directorate-General for Research and Innovation, 28 April 2017). Breeders have a wealth of experience in developing new varieties and using conventional breeding approaches for removing unwanted off-types from the final plant product that may be attributable to off-target edits. Lines 352-353: EuropaBio agrees that the analysis of potential off-target edits is of limited value for plants developed using SDN and ODM approaches. EuropaBio suggests that EFSA clearly conclude that no specific data generation to address off-target edits are needed for SDN and ODM applications. Furthermore, in the draft EFSA Scientific Opinion on Synthetic Biology developments in Plants, molecular characterisation (MC) and environmental risk assessment (ERA) aspects, EFSA indicates that "In addition, back crossing steps following DNA modifications may allow removal of most of these potential off-targets from the final product assuming they are not genetically linked to the target site" (Line 356). This text should also be included here.	The GMO Panel thanks for the comment. Regarding comment to lines 352-353, the aspect related to backcrossing steps is dealt with in section 3.2.2.2.2.	214
Logos Environmental	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	This section needs to be entirely revised to be in line with recent publications. Several key publications in this subject are absent from this opinion, e.g. Agapito-Tenfen et al. (2018) Front. Plant. Sci. 9: 1874; Cotter et al. (2020) www.testbiotech.org/en/content/rages-subreport-new-genetic-engineering-technologies; Eckerstorfer et al. (2019). Front. Bioeng. Biotechnol. 7: 31; Kawall (2019) Front. Plant Sci. 10, 525; Wolt et al. (2016) Plant Genome 9: 1 8; Zhu et al. (2017) Trends Plant Sci. 22: 38–5. These reference need to be considered as part of EFSA's deliberations. Particularly worrying here is the phrase "SDN-1 and SDN-2 approaches …produce only a fraction, if any of all the unintended genomic alterations introduced by conventional breeding". This statement is wholly without scientific basis. It is simply not true considering all the publications on off target and unintended on-target alterations, unintended incorporation of plasmids (duplicates and fragments thereof), deletions and rearrangements of sections of the genome. I am not even sure that sufficient publications exist to make this statement, as the detailed genomic sequencing necessary has been performed in only a very few, if any, plants. The statement "off target effects in SDN- and ODM-based technologies is negligible compared to conventional breeding. the GMO panel considers that the analysis of potential off target effects would be of very limited value for the risk analysis." Almost every review of genome editing considers off target effects to be of paramount importance with regard to genome editing (Agapito-Tenfen et al. (2018) Front. Plant. Sci. 9: 1874; Cotter et al. (2020) www.testbiotech.org/en/content/rages-subreport-new-genetic-engineering-technologies; Eckerstorfer et al. (2019). Front. Bioeng.	To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references.	215

		Biotechnol. 7: 31; Kawall (2019) Front. Plant Sci. 10, 525; Wolt et al. (2016) Plant Genome 9: 1 8; Zhu et al. (2017) Trends Plant Sci. 22: 38–5). Even developers acknowledge that off-target errors are important, so must EFSA. One problem that EFSA does identify, is the lack of reliable software to predict of off-target effects. The conclusion EFSA must come to is that these off-target effects could be important in terms of food/deed and environment safety and that it is not possible to evaluate such effects for a risk analysis without rigorous protocols, which need to be validated before use in the risk assessment.		
Association Française de Biotechnologies Végétales	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	AFBV edit and comments: Line 340: After "occurring" insert "in the case of spontaneous mutations or". Off-targets. We agree with EFSA that off-target changes induced by the application of SDN-1, SDN-2, SDN-3 or ODM approaches are fewer than those occurring after spontaneous mutation or with conventional mutagenesis techniques. Breeders have been dealing for decades with such changes through backcrossing.	The GMO Panel thanks for the comments. The text has been revised accordingly.	216
Julius Kühn-Institut	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	L331: delete "a". L343: replace "will" by "can be used to". It is not mandatory that backcrossing will successfully remove an off-target in any case. L360-364: Omit "(i.e. DNA free methods)" in line 361. DNA-free methods cannot result in integrations of exogenous DNA. SDN-2 will use a DNA-template (except base editing if considered equivalent). The references by Clasen, Norris, and Solomon employed templates/plasmids and hence cannot be considered DNA-free.	The GMO Panel thanks for the comment. The text has been amended to reflect the content and conclusions of the EFSA opinion on SDN-3.	217
Associazione Luca Coscioni per la libertà di ricerca scientifica	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Concerning off-targets mutations, ALC and SD agree with EFSA that the number of off-target mutations are generally far lower in number in the case of SDN-1, SDN-2 or ODM as compared to those derived from conventional mutagenesis. It is now clear that traditionally used physical and chemical mutagenesis generally produce off target mutations more relevant in number and size than those produced by SDN-1 or SDN-2. In this respect, we want to recall the sentence on the Case C-528/16 of the ECJ that considered the wide off-target mutations obtained by physical and chemical mutagenesis safe since they have a "long safety tradition". As mentioned before, traditional mutagenesis techniques are much more disruptive and therefore it is not scientifically acceptable to consider them safer than the eventual off-target mutations generated by SDN 1 or 2 or ODM.	The GMO Panel thanks for the comment.	218
Euroseeds	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Line 336/337: we suggest to reference more recent literature which shows that the technology is evolving very quickly and the need to keep up and assess the emergence of the technology and its improvement on a regular basis to be able to appropriately address potential risks (10) . Line 347/349: EFSA's position, that the risk of off-target effects for ODM is negligible, is supported by the European Commission (2017) (11) whose scientific advisers concluded that, based on the characteristics of the ODM technique, off-target effects are not expected. It is perhaps worth noting that in the paper by Modrzejewski et al. (2019) (12) , the authors suggest a relationship between the number of papers investigating off-target effects and the susceptibility of the technique to off-target effects. Line 354: we suggest including language from the draft EFSA Scientific Opinion on Synthetic Biology	Regarding comment to lines 336-337, an additional reference has been added to the text in order to support the statement that genome editing is a fast-evolving scientific field. Regarding comment to lines 347-349, the GMO Panel thanks for the comment. Regarding comment to line 354, the GMO Panel does not consider	219

		developments in Plants, molecular characterisation (MC) and environmental risk assessment (ERA) aspects. Line 356: "In addition, back crossing steps following DNA modifications may allow removal of most of these potential off-targets from the final product assuming they are not genetically linked to the target site." In addition, Euroseeds would like to highlight how the SDN-1/2 technologies are integrated into the process of conventional breeding. Plant breeding is often said to be a process not (only) of selection, but (foremost) of elimination. Any off-types, unstable lines, or lines showing characteristics such as significant differences in nutrient content, detrimental responses to environmental stresses, diseases, or the presence of other undesirable traits are discarded as soon as they are identified. The generation of genome-wide variation is part and parcel to traditional plant breeding whether by crossing or induced mutation (e.g. with SDN-1, SDN-2 and ODM). This is addressed within well-established practices for crop improvement and by breeders' best practices which document relevant phenotypic characteristics (13) . "Therefore, while process-based considerations and characterization of genome level effects may prove somewhat useful in the problem formulation for a given case of genome editing, the nature of the derived product would seem the stronger focus for any subsequent risk/safety assessment which may be conducted" concludes Wolt in his publication on "Current risk assessment approaches for environmental and food and feed safety assessment"(14) . Line 358: we suggest to include the conclusion on the same issue from the draft EFSA draft Scientific Opinion on Synthetic Biology developments in Plants, molecular characterisation (MC) and environmental risk assessment (ERA) aspects, because it has the same relevance for SDN-1, SDN-2 and ODM applications: "Therefore, taking into account all of the above, the GMO Panel considers that the analysis of potential off-targets on a regular basis would be of very limited value for the risk analysis." (10) "Base Editing: The Ever Expanding Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Tool Kit for Precise Genome Editing in Plants"; Genes 2020, 11, 466; doi:10.3390/genes11040466 (11) EUROPEAN COMMISSION (2017). New techniques in agricultural biotechnology. CEU. SAM_ADV, Directorate-General for Research and Innovation, 28 April 2017. (12) MODRZEJEWSKI, D., HARTUNG, F., SPRINK, T., KRAUSE, D., KOHL, C. & WILHELM, R. 2019. Environmental Evidence, 8. Of the 252 papers investigating off-target effects identified by Modrzejewski et al. 228 relate to CRISPR whereas only 9 studies relate to TALENs, 4 relate to ZFN, 1 relates to Meganucleases and 1 relates to ODM. The susceptibility to off-target effects varies very significantly within the various SDN techniques with the available information for ODM being in the same order as for TALENs and ZFN, and identical to Meganucleases. In this context it appears disproportionate for EFSA to single out ODM. (13)The role of conventional plant breeding in ensuring safe levels of naturally occurring toxins in food crops, Trends in Food Science & Technology 100 (2020) 51–66; https://doi.org/10.1016/j.tifs.2020.03.042 (14) Current risk assessment approaches for environmental and food and feed safety assessment, Transgenic Res (2019) 28:111–117 https://doi.org/10.1007/s11248-019-00140-7	necessary to use the same wording as in the opinion on Synthetic biology. On this aspect, the two opinions report the same conclusion. Regarding comment to line 356, the GMO Panel thanks for the comment. Regarding comment to line 358, the GMO Panel does not consider necessary to use the same wording as in the opinion on Synthetic biology. On this aspect, the two opinions report the same conclusion.	

SETA (Science and Technology in Agriculture)	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Lines 340 and following: SETA wishes to comment on off-target mutations. EFSA states that the number of off-target mutations will often be much less in the plants derived from SDN-1, SDN-2 or ODM technology as compared to those derived from conventional mutagenesis (and those are described by the EU Court of Justice as "having a long history of safe use"). SETA wishes to underline that not only the number of off-target mutations is lower, but also the extent, the relevance (including also gene regulative regions), the risks and the potential adverse effects in chemical or X-ray mutagenesis are far higher. And despite this, the EUJC on July25th, 2018 considered these mutations of "safe use". Thus, those derived from SDNs or ODM should be considered at least as equally safe.	The GMO Panel thanks for the comment. Please note that the text of the opinion has been revised to better clarify the Panel position on the off-targets aspect.	220
VIB	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Section 4.2.2 of the EFSA opinion on SDN-3 is not applicable to plants developed by SDN-1, SDN-2 or ODM approaches where those approaches have not resulted in the integration of plasmid DNA or other DNA at non-target positions. One should also not make the mistake to base possible future decisions on regulatory scope on comparability and/or applicability of certain parts of a GMO based risk-assessment approach without taking into account the comparability and/or applicability of the approaches applied to conventionally bred organisms.	The GMO Panel thanks the organization for the comment and takes note of it.	221
Union Française des Semenciers	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	-Line 354: UFS suggests to add talking points from the draft EFSA Scientific Opinion on Synthetic Biology developments in Plants, molecular characterisation (MC) and environmental risk assessment (ERA) aspects. For instance, add on line 356: "In addition, back crossing steps following DNA modifications may allow removal of most of these potential off-targets from the final product assuming they are not genetically linked to the target site." Moreover, UFS underlines that SDN-1/2 or ODM technologies would only be a part of a classical breeding process to induce genome-wide variations, as crossings or random mutagenesis do. Subsequently, by definition, with a goal to develop newly improved and safe varieties on various characteristics, a breeding programme includes many steps of elimination of undesired plants. Off-types, unstable lines, detrimental individual plants or lines showing impaired nutrient content, negative responses to environmental stresses, diseases, or any other undesirable trait are discarded as soon as they are identified. This is taken into account by breeders within long-established and best practices for crop improvement, including records of the relevant phenotypic characteristics (2). "Therefore, while process-based considerations and characterization of genome level effects may prove somewhat useful in the problem formulation for a given case of genome editing, the nature of the derived product would seem the stronger focus for any subsequent risk/safety assessment which may be conducted" concludes Wolt in his publication on "Current risk assessment approaches for environmental and food and feed safety assessment" (3). -Line 358: On the same topic, UFS suggests to take into account, and possibly include the conclusion from the draft EFSA draft Scientific Opinion on Synthetic Biology developments in Plants, molecular characterisation (MC) and environmental risk assessment (ERA) aspects "Therefore, taking into account all of the above, the GMO Panel considers that the analysis of potential off-targets on a regular basis would be of very limited value for the risk analysis.". It has the same relevance for SDN-1, SDN-2 and ODM applications. (2) The role of conventional plant breeding in ensuring safe levels of naturally occurring toxins in food	The GMO Panel thanks for the comment. Please note that the text of the opinion has been revised to better clarify the Panel's position on the off-target aspect.	222

		crops, Trends in Food Science & Technology 100 (2020) 51–66; https://doi.org/10.1016/j.tifs.2020.03.042 (3) Current risk assessment approaches for environmental and food and feed safety assessment, Transgenic Res (2019) 28:111–117 https://doi.org/10.1007/s11248-019-00140-7		
Plant Biotechnology Society	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	see attached file	The GMO Panel took note of the comment.	223
Scientific Committee for GM food and Feed, Advisory Body, Czech Republic	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	It is stated that "In general, the application of SDN-1, SDN-2,and ODM approaches result in the precise and intended modification of predetermined plant genomic sequence(s)." But this does not apply to SDN-1 which is based on random modification of predetermined plant genomic sequence(s). Not only the potential off-target mutations, but also intended on-target modifications are comparable with mutations caused by conventional plant breeding methods. So there is n oreason to treat on- and off-target mutations differentially (optimally both types should be treated as mutations exploited by conventional breeding approaches).	The GMO Panel thanks for the comment. The sentence has been revised to better reflect the different outcomes. The GMO Panel thanks for the comment. The section is mainly related to the modification to the genomic sequence in loci other than the intended ones.	224
European Coordination Via Campesina	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	This section needs to be entirely revised to be in line with recent publications. Several key publications in this subject are absent from this opinion: https://www.gmwatch.org/en/news/latest-news/19223 These reference need to be considered as part of EFSA's deliberations. Particularly worrying here is the phrase "SDN-1 and SDN-2 approaches …produce only a fraction, if any of all the unintended genomic alterations introduced by conventional breeding". This statement is wholly without scientific basis. It is simply not true considering all the publications on off target and unintended on-target alterations, unintended incorporation of plasmids (duplicates and fragments thereof), deletions and rearrangements of sections of the genome. The statement "off target effects in SDN- and ODM-based technologies is negligible compared to conventional breeding. the GMO panel considers that the analysis of potential off target effects would be of very limited value for the risk analysis." Almost every review of genome editing considers off target effects to be of paramount importance with regard to genome editing (Agapito-Tenfen et al. (2018) Front. Plant. Sci. 9: 1874; Cotter et al. (2020) www.testbiotech.org/en/content/rages-subreport-new-genetic-engineering-technologies; Eckerstorfer et al. (2019). Front. Bioeng. Biotechnol. 7: 31; Kawall (2019) Front. Plant Sci. 10, 525; Wolt et al. (2016) Plant Genome 9: 1 8; Zhu et al. (2017) Trends Plant Sci. 22: 38–5). Even developers acknowledge that off-target errors are important, so must EFSA. One problem that EFSA does identify, is the lack of reliable software to predict of off-target effects. The conclusion EFSA must come to is that these off-target effects could be important in terms of food/deed and environment safety and that it is not possible to evaluate such effects for a risk analysis without rigorous protocols, which need to be validated before use in the risk assessment.	To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references.	225
National Food Institute,	3.2.2.2.2 Alteration	It is indeed an important conclusion to make that SDN-1/SDN-2 only produce a fraction of the unintended genomic alterations introduced by conventional breeding, meaning that analysis of	The GMO Panel thanks for the comment.	226

Technical University of Denmark	elsewhere in the genome [Section 4.2.2]	potential off-targets would be of very limited value. It is a very important note that it needs to be demonstrated that the final product does not contain any of the exogenous DNA used to generate the modification.		
COST Action CA18111 - Plant genome editing – a technology with transformative potential (PlantEd)	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Line 336/337: PlantEd suggests adding literature that contends that technology is evolving very quickly and the need to keep up and assess the emergence of the technology and its improvement on a regular basis to be able to appropriately address potential risks (see, e.g., Deng et al. 2019). Line 347/349: PlantEd suggests referring to the report on "new techniques in agricultural biotechnology" prepared for the European Commission: https://ec.europa.eu/info/publications/new-techniques-agricultural-biotechnology_en. Lines 340-358: PlantEd encourages EFSA to further elaborate on the fact that, just as for conventional mutagenesis (randomly induced in vivo and in vitro mutagenesis), subsequent generations of backcrossing will allow the removal of most potential off-target mutations. In any case, the number of off-target mutations will often be much less in the plants, and their derived products, developed using SDN-1, SDN-2 or ODM technology in comparison with those developed with conventional mutagenesis. In addition, it is important to note that these site-directed nuclease technologies are an integrated part of a larger breeding process, where off-types, unstable lines, and lines displaying unwanted phenotypes are regularly sorted out and eliminated. Line 358: PlantEd suggests to include the conclusion on the same issue from the draft EFSA draft Scientific Opinion on Synthetic Biology developments in plants, molecular characterization (MC) and environmental risk assessment (ERA) aspects, because it has the same relevance for SDN-1, SDN-2 and ODM applications: "Therefore, taking into account all of the above, the GMO Panel considers that the analysis of potential off-targets on a regular basis would be of very limited value for the risk analysis."	The GMO Panel thanks for the comments. Please note that the text of the opinion has been revised to better clarify the Panel's position on the off-target aspect. The GMO Panel thanks for the comment. The SAM report on new techniques in agriculture biotechnology has been cited in the opinion. Please also note that the text of the opinion has been revised to better clarify the Panel's position on the off-target aspect. Regarding comment to lines 340-358, the GMO Panel revised the text and considers that these concepts are sufficiently elaborated in the section of the opinion. Regarding comment to line 354, the GMO Panel does not consider necessary to use the same wording as in the opinion on Synthetic biology. On this aspect, the two opinions report the same conclusion.	227
French agency for Food, Environmental and Occupational Health & Safety (Anses)	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Page 10, lines 331-333: "In addition, some Base Editing systems have been shown to present a Cas9-independent off-target effects...": please remove the capital letters on "Base Editing" (the same goes in lines 333, 335 and 337) and replace "effects" by "effect" (singular form). Page 10, line 334: "that can be either predictable (for SDN-1 and SDN-2)": please add references that support this statement. Page 10, lines 340-345: "In the EFSA opinion on SDN3, the GMO Panel concluded that the off-target changes [...] are fewer than those occurring with conventional mutagenesis techniques [...]. In addition, backcrossing following the transformation process will remove these potential off-targets from the final product, except for those that are genetically linked to the intentionally modified locus (Hahn and Nekrasov, 2019).":	The GMO Panel thanks for the comment. The text has been amended accordingly. Regarding comment to line 334, a reference has been added to support the statement with more scientific evidences. Regarding the comment for line 340-345, please note that lines 340-345 refer to the conclusions	228

		1) Same comment as on lines 89-92. 2) The case of the non-sexually propagated crops is not mentioned here, whereas it was considered in lines 251-254 (see the comment on these lines). 3) The possibility of a genetical link between the off-target changes and the intentionally modified locus is first mentioned here, which is not logical. Because of all the above considerations, the information given about the off-targets should be checked and made consistent throughout the document. Page 10, line 349: The reference Modrzejewski et al. (2019) is not relevant, because this systematic-like review reported 252 studies in which off-target effects were assessed, among which more than 90 % were conducted with CRISPR/Cas9 and only 1 with ODM. Page 10, line 352: "Therefore, because off-target effects in SDN- and ODM-based approaches is negligible...": same comment as on lines 89-92. Page 10, lines 353-354: Regarding possible off-targets, the only exhaustive way to detect them is the whole genome sequencing (WGS) of the host before and after genome editing. Indeed, the off-target changes introduced by genome editing will be similar to those that occur naturally. The comparison of the WGS before and after genome editing is therefore the best way to discriminate between the natural SNPs and those which were introduced as off-targets. The WGS will have to be performed with a coverage related to the ploidy of the considered GMP, followed by a targeted analysis of potential off-target sites. This approach applies especially for the introduction of point mutations. In the case of an insertion/deletion, targeted sequencing will certainly be sufficient. Technologies to document possible off-target effects of CRISPR have evolved over the years. We can assign a probability of "off-target" cut to more or less degenerated sites in relation to target sites (with coefficients in function of the position of the mismatches and their number). At the beginning, there were a whole series of technical variations to inspect in a targeted way the 100 to 1000 most likely sites. Right now, given the steady progress in the sequencing technique, the proper approach is total sequencing. But the relevance of this depends a lot on the species, or even the variety, its level of ploidy and the procedure used to perform the genome editing. Taking into account these considerations, Anses disagrees with the sentence "the analysis of potential off-targets would be of very limited value for the risk analysis" and considers that the identification of the off-targets should be performed according to the methodology described above. This is necessary to identify potential new hazards and risks resulting from off-targets, which are poorly documented and about which there can therefore not be any scientific consensus at this time. Additionally, research efforts should be put on the development of methods and tools allowing the identification of off-targets even in the most complex cases. Page 10, line 360: "foreign DNA": the term "exogenous DNA", which is defined in the glossary, should be preferred. Page 11, lines 368-370: "Because of all the above considerations, the GMO Panel concludes that the section 4.2.2 of the EFSA opinion on SDN3 (“Alteration elsewhere in the genome”) is applicable to plants developed by SDN-1, SDN-2, and ODM approaches.": again, Anses disagrees with this statement, because the question of the off-targets is set aside too quickly.	as in the opinion on SDN-3. In order to clarify this, the correct reference has been added (EFSA 2012). Regarding the comment for line 349, the reference Modrzejewski et al. (2019) demonstrate that indeed very few publications on off-targets are available for ODM technology. Regarding the comments for line 352 and 353-354, the GMO panel evaluated not only review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. This is the main reason why the GMO Panel considers the analysis of the off-target mutations not necessary on a regular basis. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references. Regarding comment to line 360, the text has been modified accordingly. Regarding comment to line 368-370, the GMO Panel refers the contributor to the previous response regarding the off-targets. Please also note that section 3.2.2.2.2 has been revised in order to clarify the GMO Panel’s position on the off-targets aspect.	

Corteva Agriscience	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	It is clear that in this section the EFSA GMO Panel has considered the type and frequency of off-target changes that occur with the application of both conventionally and SDN technologies and correctly concluded that "because off-target effects in SDN- and ODM-based approaches is negligible compared to conventional plant breeding, the GMO Panel considers that the analysis of potential off-targets would be of very limited value for the risk analysis". As much intentional and unintentional misinformation circulates regarding this point we think it is important that the EFSA GMO Panel has made such a clear statement to help to combat misinformation and mis-representation of off-target mutations as a unique, inevitable, abundant, and unmanageable feature of genome editing technology. Lines 327-328: "because of the potential off-target activity associated with these applications". It is important to clarify that off-target activity is not inevitable. Lines 337-339: Important to also call-out guide RNA design (1) and RNP delivery (2) as other methods to reduce the potential for off-target cutting. 1. Young J. et al. (2019) CRISPR-Cas9 editing in maize: systematic evaluation of off-target activity ad its relevance in crop improvement. Nature Scientific Reports 9: 6729. 2. Svitashev S. et al (2016) Genome editing in maize directed by CRISPR-Cas9 ribonucleoprotein complexes. Nature Communications doi: 10.1038/ncomms13274. Lines 345-347: Conclusion that "[t]he GMO Panel considers that the same conclusions remain valid also for plants generated by the application of both SDN-1 and SDN-2 approaches since they produce only a fraction, if any, of all the unintended genomic alterations introduced by conventional breeding" further supports the position that these plants should not be GMOs under the Directive since these plants contain sequences "in which the genetic material has been altered in a way" that does "occur naturally by mating and/or natural recombination could occur via conventional breeding." Lines 352-354: we support EFSA's conclusion about a very limited value of potential off-target effects for risk analysis. The document appropriately identifies the reasons for that (in context of the inherent plasticity of plant genomes and mutations introduced through conventional mutagenesis, ability to segregate by backcrossing, bioinformatic tools allowing for off-target prediction and hence appropriate design of SDN tools to mitigate off-target cutting). Lines 359-367. It is clear that in order for a plant to be able to fit under the definition included in lines 207-209 ("the application of SDN-1, SDN-2, and ODM methods result either in a random (SDN-1) or 207 in an intended (SDN-2 and ODM) modification of a targeted genomic locus without the insertion of foreign DNA" (in bold by Corteva)) companies will need to confirm that there is no "unintended insertion of plasmid DNA or other foreign DNA" in the final product. Therefore, the use of "off-target position" or "on-target position" terminology in respect to unintended insertion of plasmid DNA is confusing; furthermore, such insertions outside the target DSB site are not limited to locations sharing high degree of homology with the target sequence (potential off-target cutting sites) but can also happen at random genomic locations. We suggest simplifying this paragraph to address the unintended DNA insertion topic without association with on-target or off-target mutation sites, as being irrelevant neither for the fact of the insertion itself, nor for the off-target cutting topic. This will allow for a more focused consideration about the unintended DNA insertion topic.	The GMO Panel thanks for the comment. Regarding the comment for lines 327-328, the GMO Panel already stated that different approaches have been developed to reduce the off-target activity. An additional reference has been cited in order to support this statement. Regarding comment for line 337-339, the text has been improved accordingly. Regarding comments for lines 345-347, and 353-354, The GMO Panel thanks for the comments. Regarding the comment for lines 359-367 and 360-363, the text has been revised to improve clarity.	229

		Lines 360-363: Unintended integration of exogenous DNA with "i.e. DNA free methods" is a superficially conflicting statement unless studying the cited Andersson et al paper which suggested that the DNA source from those RNP experiments had been DNA template remnants from in vitro transcription and the chromosomal DNA. The Clausen paper is referenced as a source of "unintended on-target insertion of exogenous DNA", however PCR data provided there illustrate the fact of insertions but unable to point out where those insertions were located. Overall, this paragraph about the unintended insertion of plasmid DNA or other foreign DNA will greatly benefit from simplification and clarifications, decoupling from off-target topic and by generally noting that unintended DNA integration can happen at various genomic locations and with both DNA-based and DNA-free methods. Confirmation of absence of unintentionally integrated plasmid DNA (or other foreign DNA) is a critical part of molecular characterization regardless of where such insertion could have potentially occurred.		
Corteva	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Suggest to add reference to a recently published paper: Off-target changes in plant genome editing Nathaniel Graham, Gunvant Patil, David M Bubeck, Raymond C Dobert, Kevin C Glenn, Annie T Gutsche, Sandeep Kumar, John A Lindbo, Luis Maas, Gregory D May, Miguel E Vega-Sanchez, Robert M Stupar, Peter L Morrell Plant Physiology May 2020, pp.01194.2019; DOI: 10.1104/pp.19.01194 http://www.plantphysiol.org/content/early/2020/05/26/pp.19.01194	To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references.	230
European Plant Science Organisation, EPSO	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Line 363: The references made to Clasen et al. (2016), Norris et al. (2020) and Solomon (2020) does not refer to proper DNA-free methods since they utilize plasmid vectors to introduce the TALEN nucleases. A DNA-free delivery method cannot result in the integration of exogenous DNA but relies on careful purification of the delivered riboprotein complexes or nucleases. We suggest replacing "(i.e. DNA free methods)" by "(i.e. transient transformation methods)".	The GMO Panel thanks for the comment. The text has been amended and the expression "(i.e. DNA free methods)" has been removed.	231
Nature et Progrès Belgique	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	This section needs to be entirely revisedto Front.Bioen be in line with recent publications. Several key publications in this subject are absent from this opinion, e.g. Agapito-Tenfen et al (2018) Front. Plant.Sci. 9: 1874; Cotter et al (2020) www.testbiotech.org/en/content/rages-subreport-new-genetic-engineering-technologies; Eckerstorfer et al (2019) Front Bioeng. Biotechnol 7: 31; Kawall (2019) Front. Plant Sci.10, 525; Wolt et al (2016) Plant Genome 9:1 8; Zhu et al (2017) Trends Plant Sci. 22:38-5. These references need to be considered as part of EFSA délibérations. Particularly worrying is the phrase "SND-1 and SND-2 approaches… produce only a fraction, if any of all the unintended genomic alterations introduced by conventional breeding". This statement is wholly without scientific basis. It is simply not true considering all the publications on off target and	To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present evidences that the off-target mutations potentially generated by the application of SDN-based	232

		unintended on-target alterations, unintended incorporation of plasmids (duplicates and fragments thereof), deletions an rearrangements of sections of the genome. I ma not even sure that sufficient publications exist to make this statement, as the detailed genomic sequencing necessary has been performed in only a very few, if any, plants. The statement "off target effects in SDN-and ODM-based technologies is negligible compared to conventional breeding. The GMO panel considers that the analysis of potential off target effects would be of a very limited value for the risk analysis". Almost every review of genome editing considers off target effects to be of paramount importance with regard to genome Editing (Agapito et al (2018) Front.Plant.Sci. 1874; Cotter et al (2020) www.testbiotech.org/en/content/rages-subreport-new-genetic-engineering-technologies; Eckerstorfer et al (2019) Front. Bioeng. Biotechnol. 7:31; Kawall (2019) Front.Plant. Sci. 10, 525; Wolt et al (2016) Plant Genome 9:1 8; Zhu et al (2017) Trends Plant Sci. 22:38-5. Even developpers aknowledge that off-taget errors are important, so must EFSA. One problem that EFSA identify is the lack of reliable software to predict off-target effects. The conclusion EFSA must come to is that these off-target effects could be important in terms of food/feed and environmental safety and that it is not possible to evaluate such effects for a risk analysis without rigorous protocols, which nee to be validated before use in the risk assessment.	methods for genome editing are of the same type as those produced by conventional breeding including random mutagenesis. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references.	
Haut Conseil des biotechnologies (High Council for Biotechnology)	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	l. 342. "conventional mutagenesis techniques that have been used previously and have a long history of safe use". Suggestion to delete "that have been used previously and have a long history of safe use" since it is supposed to be part of the definition of conventional breeding techniques. An alternative would be to use ", which" instead of "that". In fact, what is often overlooked and could be made clearer is that the long history of safe use of conventional mutagenesis techniques is associated to products resulting from a stringent selection following the mutagenesis. l. 342-345. "backcrossing following the transformation process will remove these potential off-targets from the final product (…)" We recommend replacing "will" by "may", and add: "Backcrossing, however, is not commonly used for non-sexually propagated plants and not practical for plants with particularly long generation time such as most trees." l. 350-352. Considering the current state of knowledge, it seems difficult to extrapolate to ODM what has been observed with SDN approaches. The absence of DSB in ODM is a significant difference with SDN approaches. More work should be done regarding the possibility that ODM results in off-target modifications and how to anticipate and/or identify them. Without further information, however, we agree that the potential for off-target effects should be envisaged for ODM. See below regarding the conclusions to draw from the presence of possible off-target changes. l. 352-354. It does not seem absurd nor excessive to ask for information on off-target effects in the risk assessment of plants generated by SDN and ODM-approaches, especially considering that the stated comparison is not relevant for plants whose genetic improvement may not be commonly based on conventional mutagenesis techniques that generate more mutations than these new techniques. Sanchez-Leon et al. (2018), for example, have assessed the possibility for off-target activity to affect coding regions in wheat. This type of analysis could be systematically asked from applicants to ensure potentially remaining mutations in the plants may not be associated with a predictable risk.	Regarding comment for line 342, the text refers to the conclusions of the EFSA opinion on SDN-3. To better clarify this, the citation has been inserted at the end of the statement. Regarding the comment for line 342-345, please note that lines 340-345 refer to the conclusions as in the opinion on SDN-3. In order to clarify this, the correct reference has been added (EFSA 2012). Regarding the comment for line 350-352, please note that the entire section has been revised and the previous conclusion on the off-target effects for ODM has been removed. Regarding the comments for lines 352-354 and 354-358, the GMO panel evaluated not only review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their	233

		l. 354-358. The current limitation in plant genomic reference sequence data does not seem a valid justification for not asking for an analysis of off-target modifications and associated risk assessment, especially in cases where backcrossing is not feasible or practical to remove unintended mutations. The analysis will improve with the availability of better tools and more sequence data. l. 360-364. The sentence is unclear and would deserve some clarification. Clarify which exogenous DNA is meant. Again, there may be some confusion with SDN-2-mediated insertion/generation of a mutated sequence. l. 364-367. We agree with this requirement, which should also apply following removal of the SDN module after a stable integration. It should also be clarified in that section that stable integration of the SDN module may be intentional. This is also an alteration elsewhere in the genome than in the site targeted for the mutation. In that case, risk assessment associated with this transgenic cassette should be carried out as planned in the corresponding EFSA guidance. l. 368-370. We would support asking for the systematic assessment of off-target effects in SDN and ODM-generated plants. Off-target effect assessment would essentially consist in three steps: (1) predictive analysis based on (i) off-target activity of the nuclease considering the delivery method (for SDN approaches) and on (ii) sequence homology, in the recipient genome, to the guide RNA for SDN-approaches or to the oligonucleotide for ODM, (2) verification of potential modifications at these predicted sites in the sequence of the final product, (3) risk assessment of the identified sequence changes. Regarding ODM technique, we also note that more work should be done regarding the possibility for off-target modifications and how to anticipate and search for them. Furthermore, further analysis should be required in cases where an active SDN module, stably integrated, has not been removed from the final product, considering that the system may generate additional modifications (1) during deliberate release of the final product depending on conditions (e.g. prolonged or higher expression of the nuclease may induce more off-target changes), and (2) following hybridization with, or crossing into, another genotype with potentially different off-target sites. These recommendations should also apply to SDN-3. Sanchez-Leon, S., Gil-Humanes, J., Ozuna, C.V., Gimenez, M.J., Sousa, C., Voytas, D.F., and Barro, F. (2018). Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. Plant Biotechnol J 16, 902-910.	analysis. These papers present 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. This is the main reason why the GMO Panel does not consider an analysis of off-target mutations necessary on a regular basis. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references. Regarding the comment for lines 360-364, the text has been revised to improve clarity. Regarding the comment for line 364-367, the GMO Panel considers that the text of the opinion delivers the same message proposed in the comment. Nevertheless, the text has been revised to improve clarity. Regarding comment for line 368-370, the GMO Panel reiterates its position. To develop the opinion, the GMO panel evaluated not only review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. This is the main reason why the GMO Panel	

			does not consider an analysis of off-target mutations necessary on a regular basis. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references. Please note that according to the terms of reference of the mandate, the SDN-3 approach was not included.	
Testbiotech	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	[Line 331, after first bullet, add text:] "Specific off-target effects caused by SDN-1 or SDN-2 have been detected and reported during experiments with several crop plants, including rice, soy and barley (Modrzejewski et al., 2019; Wolt et al., 2016; Zhu et al., 2017; Eckersdorfer et al., 2019). Braatz et al. (2017), e.g.showed by using whole-genome sequencing that the Agrobacterium transformation of oilseed rape with a CRISPR/Cas9 expression construct resulted in at least five independent insertions of vector backbone sequences in the genome of the modified plant." [line 344 after first bullet -370, delete and replace text:] "The GMO Panel considers that this conclusion is not sufficiently valid, at least not in the case of SDN-1 and SDN-2 applications. The set of data needed for risk assessment will be dependent on a case by case basis and cannot generally be limited by criteria such as the insertion of additional genes. For example, if high amounts of biological mutagens are applied to plant cells, the number of unintended effects might increase. In this regard, the approaches using transient activity of the nucleases might need more data on the effects caused by the nuclease if compared to permanent expression based on a CRISPR/Cas-transgene. On the other hand, the insertion of transgenes might cause additional off-target effects which are not caused by the activity of the final effector. Further, in the case of serial applications or multiplexing, the number of unintended effects might also be increased due to the repeated application of the genetic engineering process. Similarly, as it is the case with unintended on-target effects (see above), in order to detect such unintended effects it is essential to apply available methods carefully to analyze the genome. Furthermore, the specific methods which were applied during the genetic engineering of an organism are known and also taken into account during risk assessment. In some cases, the data set needed to perform risk assessment of SDN-1, SDN-2 (and potentially also ODM) might go far beyond those needed for SDN-3, e.g. if many copies of one genes are altered or several genes are targeted by multiplexing To a similar extent, as is the case with unintended on-target effects in regard to environmental risks, there are several risk scenarios that need to be considered: relevant categories of examples include changes in the composition of plants that may impact the food web, changes in the composition of plants that may impact plant communication and interaction with the environment, changes in the biological characteristics of the GE organisms meant to enhance fitness and potential next generation effects of organisms with the potential to persist and propagate in the environment (see Testbiotech,	The GMO Panel takes note of the comments. To develop the opinion, the GMO panel evaluated not only review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. This is the main reason why the GMO Panel does not consider an analysis of off-target mutations necessary on a regular basis. Nevertheless, the GMO Panel has revised the text of the section in order to improve clarity, including some additional relevant references.	234

		2020). Also in regard to unintended off-target effects, there are several publications showing the range of aspects that have to be taken into account in regard to the safety of gene products and organisms derived from SDN-1 and SDN-2 processes (for overview, see Agapito-Tenfen et al., 2018, Eckerstorfer et al., 2019; Testbiotech, 2020, Cotter et al., 2020; Kawall et al., 2020). While an increasing number of publications have investigated off-target effects for SDN-based technologies, information on the off-target mechanism and frequency for ODM is quite limited (Modrzejewski et al., 2019; Eckersdorfer et al., 2019). Due to the lack of information, the Panel found it difficult to reach to any conclusions. It should also be taken into account that, although some biochemical and bioinformatic tools are available for off-target prediction (Bae et al., 2014, Tsai et al., 2015, Cameron et al., 2017, Peng et al., 2018), the limited availability and/or completeness of plant genomic sequences and their intra-species and intra-varietal variability would in many cases not allow for a reliable prediction of potential off-target mutations. Because of all the above considerations, the GMO Panel concludes that the section 4.2.2 of the EFSA opinion on SDN3 ("Alteration elsewhere in the genome") is partially applicable, but in many cases will not be sufficient to assess the risks of plants developed by SDN-1, SDN-2, and ODM approaches."		
Sciensano	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Line 359 : "When plant transformation is used to introduce the SDN module, the unintended insertion of plasmid DNA or other foreign DNA at off-target positions can happen" => plant transformation to introduce the SDN module is not targeted, so insertion at off-target position has no meaning here.	The GMO Panel thanks for the comment. The text refers to the fact that transformation methods themselves can introduce exogenous DNA in the plant genome. The text has been revised to improve clarity.	235
Società Italiana di Genetica Agraria - Italian Society of Agricultural Genetics (SIGA)	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Line 343 "… these potential off-targets… " We suggest: "… these potential off-target mutations …". Lines 361-362 " … can result in the unintended on-target or off-target integration of exogenous DNA whose sequence is known a priori …" We suggest to specify the origin of this exogenous DNA insertion.	Regarding the comment for line 343, the text has been amended accordingly. Regarding the comment for line 361-362, the origin of the exogenous DNA has been clarified by including the sentence "derived from the genome editing process" as demonstrated by the cited publications.	236
Cornell University's Alliance for Science	3.2.2.2.2 Alteration elsewhere in	We agree with the finding in line 353 in which the GMO Panel considers that the analysis of potential off- targets would be of very limited value for the risk analysis.	The GMO Panel thanks for the comment.	237

	the genome [Section 4.2.2]			
GenØk-centre for biosafety	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Copied from section 3.2.2.1 above: "The repair mechanisms is not mentioned in this section of the assessment and should be mentioned to highlight that although these mechanisms are known, they vary between cell types and there is still much that is unknown about how distinct cell types work. Cas-9 proteins and other editing enzymes have the potential to create genomic instability in cases where polymerases and helicases are disrupted. These enzymes are part of the DNA replication and transcription machinery. Cells do repair these errors as well. These non-target changes in the genome is not mentioned in the draft part here, but should have a note with a focus on choice of editing system, specificity of repair mechanisms, and analysis of potential off-target effects". However, in this part of the document, EFSA considers the potential for off-target as "negligible" compared to conventional breeding and that analysis of these would therefore be of "limited value". Clearly, with the knowledge on potential for off-target effects based on genome editing system used, there should be a focus on analysis of genome-editing method used to assess whether the GM plant producer have chosen a method for editing that has a history of low frequency of non-target effects or not. Thus, such an analysis would be necessary in all cases of genome edited plant. It has been described by Young et al ("CRISPR-Cas9 Editing in Maize: Systematic Evaluation of Off-target Activity and Its Relevance in Crop Improvement", Scientific reports, 9:2019) that with an well designed gRNA, off-targeting can be minimized, thus effects causing genetic variation by Cas cleavage could potentially be neglible. An assessment of mnethods used would therefore be needed, in order to evaluate this. **Copied from the submitted pdf file:** Off-target activity cannot be reliably predictable EFSA states that the off-target activity of SDN-1 and SDN-2 are predictable (lines 333 and 334). This statement is wrong for two main reasons: 1) SDN-1 and SDN-2 approaches can use a variety of techniques, as mentioned before, and it is not clear to what techniques is EFSA referring in this statement. 2) It is not correct that in silico analysis can reliably predict off-target activities of gene editing techniques. For example, many of the CRISPR/Cas9 design tools include information about potential off-target sites in the genome of interest, but not every algorithm searches for every kind of off-target effect (e.g., DNA or RNA bulges). It has also been observed that analyses from in silico predictions are not always correct and their results don't always align because the CRISPR/Cas9 system is not completely understood7. EFSA statement even contradicts its own analysis of such softwares in lines 354-358, where it says prediction softwares are not reliable. The number of off-target mutations is not relevant for risk assessment Whereas it is logical to think that the more off-target mutations in the host genome the more probability of risk, it is not correct to think that fewer off-target changes are equivalent to a safe profile. In this regard, we suggest that EFSA deletes lines 340-342 as it does only mislead the risk assessment aspect of off-target activity of site-directed nucleases. Back-crossing does not remove all off-target changes EFSA states that "[…] backcrossing following the transformation process, will remove these potential off-targets from the final product […]" (lines 343 and 344).	To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references.	238

		It is known to any plant breeder that the main challenge in breeding is breaking linkage groups. It is not possible to remove off-target changes performed by nucleases, which overcomes linkage drag effects in plants, by simple cross8. For example, genomic analyses in tomato plants have indicated that the linkage drag associated with genome segmentation covers nearly 25.6% of the assembled genome. Therefore, this statement should be deleted and the need to verify off-target changes should be discussed even when organisms have been back-crossed with untransformed lines. Conventional breeding does not produce unintended genomic alterations The following statement from EFSA: "SDN-1 and SDN-2 approaches since they produce only a fraction, if any, of all the unintended genomic alterations introduced by conventional breeding" is false, misleading and purposeful: 1) What SDN-1 and SDN-2 techniques is EFSA referring to? Is it CRISPR? TALEN? What delivery method? DNA-free? Transgenesis? 2) Conventional breeding is not chemical or radiation mutagenesis. Therefore, it does not produce unintended genomic alterations. Conventional breeding as per GMO Directive is simple the natural cross of individuals. 3) How can a quantitative measure, such as a "fraction", informs anyone about the potential risks of a technique? Please see my comments above on the number of off-target mutations. The lack of information on off-target activity of ODM should be reported not extrapolated EFSA states "Despite the lack of information on possible off-target effects, it is reasonable to assume that the same conclusions apply to ODM since this technology is also based on sequence-specific site recognition" (lines 350-352). Again, EFSA limits its analysis of ODM off-target effects on the aspect of sequence similarity whereas the other techniques are completely different and use nucleases that can cause double-stranded breaks, not the case for ODMs. How can off-target activity of gene editing not be of value to risk assessment? EFSA states that the off-target effects of SDN-1 and SDN-2 and ODM are negligible and of limited value for the risk analysis. It is unclear why EFSA does not recognizes all the scientific literature on off-target activity of such techniques which has led to a global moratorium on all clinical uses of human germline editing9. This entire section is flawed and of no use for the assessment of ToR1. For a recent review on alterations elsewhere in the genome of gene-edited organisms, please read Agapito-Tenfen (2018)10**.**		
Kleter, Dr Gijs A.	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Item: "In addition, backcrossing following the transformation process will remove these potential off-targets from the final product, except for those that are genetically linked to the intentionally modified locus": Comment: To what extent can additional, on-target edits that are still genetically linked to the intended edit (and hence do not readily segregate from it) be identified using currently recommended strategies? For example, could DNA sequencing methods be used for this purpose, besides the cited bioinformatics? (see, for example, Weisheit et al., 2020, "Detection of deleterious on-target effects after HDR-mediated CRISPR editing", Cell Reports 31, 107689, https://doi.org/10.1016/j.celrep.2020.107689)	To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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	239

			by conventional breeding including random mutagenesis. Please consider that the possibility to eliminate potential off-target mutations from the genome of the final product by backcrossing is not the main reason why the off-target analysis is not deemed necessary on a regular basis. Moreover, the molecular characterization of the target site is still considered a mandatory requirement for the risk assessment as laid down in the EU regulation of GMO. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references.	
Federal Agency for Nature Conservation	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Lines 329-330: Please add other factors that can influence off-target activity, e.g. from Ecker-storfer et al. (2019), (1) the frequency of homologous sequences in the genome, (2) the characteristics of the specific nuclease type, (3) the expression level of the nuclease, (4) the time span for which the nuclease is present in the target cell and (5) the accessibility of the homologous sequence and of any potential off-target sequences in the chromatin, and amongst the ones from Modrzejewski et al. (2019), (6) quality of available biased detection methods and (7) methodology of SDN delivery. Lines 342-344: According to the draft, backcrossing following transformation will remove any potential off-target effects from the final product, except the ones that are genetically linked to the on-target locus. Another exception to consider and to mention is that, unlike conventional breeding, genome editing can be used to transform elite lines directly without any or much backcrossing. This is to save time which is one of the arguments put forward in favour of genome editing. Lines 347-358: As indicated the number of off-target effects in SDN interventions is not defined, but depends on some variables (see also comment to lines 329-330). Again, the case of multiplexing SDN interventions is missing but relevant, because here the number of potential off-target effects is considerably higher. We are also missing the point that efficiency and off-target activity are obviously related to each other: the longer an SDN module is active, the more efficient the genome editing can be expected to be, but in turn, also the number of off-target effects. This is one of the reasons why the outcomes of SDN interventions can vary and why they should be analysed at the molecular level. Up to now there are only a few studies that have applied Whole Genome Sequencing to investigate off-target effects of CRISPR in vivo systems (Agapito-Tenfen et al 2018; Modrzejewski et al. 2019). Data is comparably easy to obtain and should be made available for risk assessment. Alternatively, mutations may be identified via methods already established in conventional breeding for techniques such as TILLING and verified by specific sequencing. Agapito-Tenfen, Sarah Z.; Okoli, Arinze S.; Bernstein, Michael J.; Wikmark, Odd-Gunnar; Myhr, Anne I. (2018): Revisiting Risk Governance of GM Plants: The Need to Consider New and Emerging Gene-Editing Techniques. In: Frontiers in plant science 9, p. 1874. DOI: 10.3389/fpls.2018.01874.	To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. Please consider that the possibility to eliminate potential off-target mutations from the genome of the final product by backcrossing is not the main reason why the off-target analysis is not deemed necessary on a regular basis. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references.	240

		Eckerstorfer, Michael F.; Heissenberger, Andreas; Reichenbecher, Wolfram; Steinbrecher, Ricarda A.; Waßmann, Friedrich (2019): An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs). In: Frontiers in bioengineering and biotechnology 7, p. 319. DOI: 10.3389/fbioe.2019.00031. Modrzejewski, Dominik; Hartung, Frank; Sprink, Thorben; Krause, Dörthe; Kohl, Christian; Wilhelm, Ralf (2019): What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a systematic map. In: Environmental Evidence 8 (1), p. 27. DOI: 10.1186/s13750-019-0171-5.		
GMWatch	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	In the draft document, EFSA states, "SDN-1 and SDN-2 approaches …produce only a fraction, if any of all the unintended genomic alterations introduced by conventional breeding". This statement is unsupported by any scientific evidence and is contradicted by many findings of the studies cited above. Moreover, it is not possible to make such a statement, given the scant information published on the genetic sequences of plants produced using SDN-1 and -2 procedures. EFSA also states, "because off-target effects in SDN- and ODM-based technologies is [sic.] negligible compared to conventional breeding, the GMO panel considers that the analysis of potential off target effects would be of very limited value for the risk analysis." This is an extraordinary statement, given the evidence of the studies cited in the "List of studies" in the attachment, which find off-target effects, as well as unintended on-target effects, of gene editing highly important. Also, as stated above, no evidence base exists that would enable a comparison of the effects of off-target effects from gene editing and ODM technologies with the effects of conventional breeding.	The GMO Panel takes note of the comments. To develop the opinion, the GMO panel evaluated not only review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. This is the main reason why the GMO Panel does not consider an analysis of off-target mutations necessary on a regular basis. Nevertheless, the GMO Panel has revised the text of the section in order to improve clarity, including some additional relevant references	241
Envirnonmental association Za Zemiata	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	[Line 331, after first bullet, add text:] "Specific off-target effects caused by SDN-1 or SDN-2 have been detected and reported during experiments with several crop plants, including rice, soy and barley (Modrzejewski et al., 2019; Wolt et al., 2016; Zhu et al., 2017; Eckersdorfer et al., 2019). Braatz et al. (2017), e.g.showed by using whole-genome sequencing that the Agrobacterium transformation of oilseed rape with a CRISPR/Cas9 expression construct resulted in at least five independent insertions of vector backbone sequences in the genome of the modified plant." [line 344 after first bullet -370, delete and replace text:] "The GMO Panel considers that this conclusion is not sufficiently valid, at least not in the case of SDN-1 and SDN-2 applications. The set of data needed for risk assessment will be dependent on a case by case basis and cannot generally be limited by criteria such as the insertion of additional genes.	The GMO Panel takes note of the comments. To develop the opinion, the GMO panel evaluated not only review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present evidences that the off-target mutations potentially generated by the application of SDN-based methods for genome	242

		For example, if high amounts of biological mutagens are applied to plant cells, the number of unintended effects might increase. In this regard, the approaches using transient activity of the nucleases might need more data on the effects caused by the nuclease if compared to permanent expression based on a CRISPR/Cas-transgene. On the other hand, the insertion of transgenes might cause additional off-target effects which are not caused by the activity of the final effector. Further, in the case of serial applications or multiplexing, the number of unintended effects might also be increased due to the repeated application of the genetic engineering process. Similarly, as it is the case with unintended on-target effects (see above), in order to detect such unintended effects it is essential to apply available methods carefully to analyze the genome. Furthermore, the specific methods which were applied during the genetic engineering of an organism are known and also taken into account during risk assessment. In some cases, the data set needed to perform risk assessment of SDN-1, SDN-2 (and potentially also ODM) might go far beyond those needed for SDN-3, e.g. if many copies of one genes are altered or several genes are targeted by multiplexing To a similar extent, as is the case with unintended on-target effects in regard to environmental risks, there are several risk scenarios that need to be considered: relevant categories of examples include changes in the composition of plants that may impact the food web, changes in the composition of plants that may impact plant communication and interaction with the environment, changes in the biological characteristics of the GE organisms meant to enhance fitness and potential next generation effects of organisms with the potential to persist and propagate in the environment (see Testbiotech, 2020). Also in regard to unintended off-target effects, there are several publications showing the range of aspects that have to be taken into account in regard to the safety of gene products and organisms derived from SDN-1 and SDN-2 processes (for overview, see Agapito-Tenfen et al., 2018, Eckerstorfer et al., 2019; Testbiotech, 2020, Cotter et al., 2020). While an increasing number of publications have investigated off-target effects for SDN-based technologies, information on the off-target mechanism and frequency for ODM is quite limited (Modrzejewski et al., 2019; Eckersdorfer et al., 2019). Due to the lack of information, the Panel found it difficult to reach to any conclusions. It should also be taken into account that, although some biochemical and bioinformatic tools are available for off-target prediction (Bae et al., 2014, Tsai et al., 2015, Cameron et al., 2017, Peng et al., 2018), the limited availability and/or completeness of plant genomic sequences and their intra-species and intra-varietal variability would in many cases not allow for a reliable prediction of potential off-target mutations. Because of all the above considerations, the GMO Panel concludes that the section 4.2.2 of the EFSA opinion on SDN3 ("Alteration elsewhere in the genome") is partially applicable, but in many cases will not be sufficient to assess the risks of plants developed by SDN-1, SDN-2, and ODM approaches."	editing are of the same type as those produced by conventional breeding including random mutagenesis. This is the main reason why the GMO Panel does not consider an analysis of off-target mutations necessary on a regular basis. Nevertheless, the GMO Panel has revised the text of the section in order to improve clarity, including some additional relevant references.	
Corporate Europe Observatory	3.2.2.2.2 Alteration	As noticed under section 1.1, many recent publications have been ignored, e.g. Agapito-Tenfen et al. (2018) Front. Plant. Sci. 9: 1874; Cotter et al. (2020) www.testbiotech.org/en/content/rages-	The GMO Panel takes note of the comments. To develop the	243

	elsewhere in the genome [Section 4.2.2]	subreport-new-genetic-engineering-technologies; Eckerstorfer et al. (2019). Front. Bioeng. Biotechnol. 7: 31; Kawall (2019) Front. Plant Sci. 10, 525; Wolt et al. (2016) Plant Genome 9: 1 8; Zhu et al. (2017) Trends Plant Sci. 22: 38–5. These publications need to be considered in the current Opinion. The statement "SDN-1 and SDN-2 approaches …produce only a fraction, if any of all the unintended genomic alterations introduced by conventional breeding" is at odds with numerous scientific publications. In addition, the draft Opinion reads: "off target effects in SDN- and ODM-based technologies is negligible compared to conventional breeding. the GMO panel considers that the analysis of potential off target effects would be of very limited value for the risk analysis." However, off-target effects are very relevant with regard to genome editing (Agapito-Tenfen et al. (2018) Front. Plant. Sci. 9: 1874; Cotter et al. (2020) www.testbiotech.org/en/content/rages-subreport-new-genetic-engineering-technologies; Eckerstorfer et al. (2019). Front. Bioeng. Biotechnol. 7: 31; Kawall (2019) Front. Plant Sci. 10, 525; Wolt et al. (2016) Plant Genome 9: 1 8; Zhu et al. (2017) Trends Plant Sci. 22: 38–5). Off-target effects could be important in terms of food/deed and environmental safety. These need to be evaluated through a rigorous risk assessment process. The following publication summarises impacts of SDN-1 and SDN-2 techniques on the environment, for instance via changed composition of the plant and its impact on the food web: Testbiotech (2020) Overview of genome editing applications using SDN-1 and SDN-2 in regard to EU regulatory issues. www.testbiotech.org/node/2569	opinion, the GMO panel evaluated not only review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. This is the main reason why the GMO Panel does not consider an analysis of off-target mutations necessary on a regular basis. Nevertheless, the GMO Panel has revised the text of the section in order to improve clarity, including some additional relevant references.	
BUND e.V. / Friends of the Earth Germany	3.2.2.2.2 Alteration elsewhere in the genome [Section 4.2.2]	Line 326 - 327 CHANGE sentence to " In general, the application of SDN-1, SDN-2, and ODM approaches are intended to result in a more precise and foreseeable modification of predetermined plant genomic sequence(s)." Line 334 ADD after "predictable": "in parts" Line 339 ADD after "effects": ", though findings in the literature still show an extent of off-target effects not to be neglected" Line 342 ADD after "use": ", still it is important here to bear in mind that plants altered by conventional mutagenesis only are used to contribute to variety in breeding, but varieties obtained through SDN-1 or SDN-2 genomic techniques are meant to transfer exactly the one altered gene to offspring. In addition, considering the many off-target and unintended on-target alterations observed in genome editing, EFSA should provide data to support this statement, since the detailed genomic sequencing that would be necessary has been performed only rarely." Line 342 - after insertion suggested above - to line 358 (incl.) CHANGE paragraph:	Regarding comment to line 326-327, the text has been revised to improve clarity. The GMO Panel considers that for the comments related to lines 334, 339, 342, an explanation of the rational for the proposed change is lacking. Therefore, the proposed changes have not been integrated in the text of the opinion. Regarding comment to line 342-358, please note that lines 340-345 refer to the conclusions as in the opinion on SDN-3. In order to clarify this, the correct reference has been added (EFSA 2012). To develop the opinion, the GMO panel evaluated not only review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their	244

		Backcrossing following the transformation process is often referred to as a means to remove these potential off-targets from the final product, but findings in literature show that this is not always possible, especially for those off-targets that are genetically linked to the intentionally modified locus (Hahn and Nekrasov, 2019), but not limited to those (Michno et al. 2020). While an increasing number of publications have investigated off-target effects for SDN-based technologies, information on the off-target mechanism and frequency for ODM is quite limited (Modrzejewski et al., 2019). Because of the lack of information on possible off-target effects from ODM, and since more and more findings show that the use of CRISPR-Cas is not as specific as had been assumed (Eckerstorfer et al. 2019, Agapito-Tenfen et al. 2018), it seems highly recommendable to enforce a better understanding of off-target effects and increase research for off-target prediction and analysis. This has to include effects exerted by other cas enzymes such as Cas12a / Cpf1 (Murugan et al. 2020). General comment: This whole section needs revision to take into account newer findings and recent publications. Key studies and findings have been added in the changes and additions proposed above. When deciding on the levels of risk assessment of SDN-1 and SDN-2, the findings in literature on unforeseeable and unintended modifications have to be taken into account. Therefore, specific and improved risk assessments for SDN-1, SDN-2, and ODM approaches are mandatory; EFSA should adopt those conclusions as part of this opinion.	analysis. These papers present 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. This is the main reason why the GMO Panel does not consider an analysis of off-target mutations necessary on a regular basis. Please consider that the possibility to eliminate potential off-target mutations from the genome of the final product by backcrossing is not the main reason why the off-target analysis is not deemed necessary on a regular basis. Nevertheless, the GMO Panel has revised the text of the section in order to improve clarity, including some additional relevant references	
ENSSER	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	L374: As previously, please spell out if "conventional breeding techniques" is in fact meant to read 'conventionally used mutagenesis techniques'. We want to again express our concern regarding this choice of baseline and comparator. We do not deem this appropriate, neither for the 2012 opinion nor for the current 2020 draft opinion. L384-385: We regards this statement as faulty and it should not be reiterated here as the basis for this current evaluation and opinion. Please see comments Line 341, regarding "fewer". L386-387: Again, as explained in our comments earlier on, SDN-1 or SDN-2 mutations are not same type of mutations as conv breeding: all copies affected, areas otherwise less accessible to chemically and physically induced mutations seem to be more open to biologically/enzymatically induced mutations (Kawall 2019). It would be helpful if you could explain what exactly the panel understands under "type of mutation" L387-389: Whilst the DNA cleavage mechanism is of course the same for SDN1,2,3 as long as the same endonuclease is being employed, the repair mechanism though is not. SDN-1 relies on NHEJ, whilst SDN-3 relies on HDR – which commonly will occur at different cell cycle stages or in different tissue. Thus – in the presence of HDR - unintended DSBs will tend to be repaired to the original sequence if possible, though further research is needed to investigate this, whilst NHEJ will by definition make eroneous repairs, from small to large deletions, insertions, even translocations. Thus the conclusions drawn or eluded to in this paragraph, i.e. that the mutations and the mutation rate are the same for SDN-1, -2 and -3 deem flawed.	Regarding comment to line 374, the EFSA scientific opinion on SDN-3 was developed by comparing the type of outcome and mutations produced by SDN-3 to those generated by conventional breeding, including random mutagenesis. For this reason, the GMO Panel followed the same approach for SDN-1, SDN-2, and ODM, in order to be able to assess the applicability of section 4 and conclusions of the opinion on SDN-3 to plant developed via these approaches. Please note that a footnote has been inserted in the text to refer to the list of techniques relevant for a comparison as indicated in the opinion on SDN3 (section 3.2.1).	245

		Without robust scientific evidence based on empirical studies across different species and with different nucleases this whole paragraph should be withdrawn. 397-410: Bullet point number 4 fails to consider unintended modifications that are present due to the processes employed for SDN applications, namely the whole spectrum of non-target or off-target modifications (in the widest sense), also including near-target mutations. Furthermore, the opinion given in this paragraph also fails to consider unintended on-target effects, such as metabolic knock-on effects, and altered gene regulation in close-by or distant areas (depending on regulatory interactions), which can give rise to altered compound compositions in plants and plant products other than expected from the trait. Furthermore, knockout modifications (whether on-target or off-target) may give rise to truncated proteins that no longer provide their original function, but may provide an altered function, which constitutes a risk. Observations have also been made showing that knock-out frame-shift mutations can give rise to the production of new mRNAs and potentially even new proteins (Tuladhar et al. 2019). Finally, the whole draft opinion, and in particular this paragraph has failed to note and to elaborate on the fact, that SDN-1 can be used and is being used for complex interventions in the genome by either multiplexing or serial application of single SDN-1 applications. Such complex alterations and interventions will give rise not only to altered gene regulation but also to a complexity and magnitude of novel genetic combinations and traits, and accordingly risks both for the environment as well as for human and animal health. All these points just covered in response to bullet 4 need to be thoroughly studied and included in any scientific opinion on risk and risk assessment of SDN-1, -2 and ODM applications, including this draft opinion, and in particular this bullet point. Please delete the point 4 as it stands and address the issues raised here.	Regarding comment to line 384-389, To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references. The GMO Panel understands that the term "multiplexing" used in the comment may refer to the simultaneous mutation of multiple plant genomic loci. Although multiplexing approach is not specifically discussed in the opinion, the GMO Panel considers that all the considerations included in the opinion on SDN-based methods are also applicable to multiplexing approaches. Moreover, it should also be noted that multiplexing is not specific to SDN/ODM approaches as it can also be achieved by transgenic and conventional breeding approaches. The GMO Panel would also like to remind that the "case-by-case" approach can also be applied to genome edited plants. The GMO Panel is aware of the fact that a complexity of scenarios is possible due to the application of SDN-based methods. In this regard, the GMO Panel refers to the mandate on GM plant	

			generated via synthetic biology approaches.	
EuropaBio	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	Lines 400-403: EuropaBio suggests that EFSA should discuss here that using the case-by-case approach and the application of problem formulation is useful in guiding the assessment to establish what data requirements outlined in previous guidance are relevant for the product. For data requirements not relevant for a particular product the derogation clause in IR 503/2013 should be used. We also suggest that EFSA considers discussions with applicants prior and during submission to ensure that fit-for-purpose risk assessments are produced. Lines 408-409: EuropaBio agrees with this conclusion regarding the need for "less amount of experimental data" for plants generated using SDN-1, SDN-2 and ODM approaches. The need for the experimental data requested in existing EFSA guidance, in the absence of any transgene, should be guided by the problem formulation approach and will depend mainly on the phenotype, not the process used to obtain it.	The GMO Panel thanks for the comment and takes note of it.	246
Association Française de Biotechnologies Végétales	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	AFBV edit and comments: Line 383: insert ", intragene or cisgene" after "transgene". Comments on EFSA's 4th consideration beginning at Line 397: EFSA concludes that in the absence of any transgene, the amount of experimental data needed for the risk assessment will mainly depend on the modified trait introduced and even less amount of experimental data would be needed for plants produced via SDN-1, SDN-2, and ODM compared to plants generated via SDN-3. That conclusion argues for taking into account the type of edited plants which are produced as suggested by EFSA in Section 3.2.2.1. Depending on the type of edited plants, some will more closely correspond to a OGM while others are extremely similar to, and in many cases indistinguishable from, plants obtained by conventional breeding. After review of a small amount of data such plants should be excluded from the GMO legislation and regulated in the same manner as conventionally bred plants. Case-by-case flexibility for data requirements should be re-instated (as existed before 2013), as suggested by EFSA. For this reason AFBV in its proposal suggested an amendment to the Directive 2001/18/CE and GMO Regulations to exclude from all GMO legislation edited plants which can be assimilated to plants obtained through conventional breeding. Our comments are particularly justified as the GMO Panel did not identify any additional hazard associated to the use of the SDN-1, SDN-2 and ODM approaches as compared to both SDN-3 and conventional breeding techniques, including conventional mutagenesis.	Regarding comment to line 384, the text has been amended accordingly. The GMO Panel thanks AFBV for the comment and takes note of it.	247
Julius Kühn-Institut	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to	L394-395: It should be clarified that DNA-free approaches will not introduce a SDN module into the genome. L401-404: With regards to the consideration raised under item 3.:The sentence could be rewritten as " Indeed, those requirements related to the presence of transgenes are generally not relevant because of the reason outlined in point 1."	Regarding comment to lines 394-395, the GMO Panel states that "in some cases the SDN module could be stably introduced" which implies that in other cases it will not (i.e. in case of DNA-free delivery).	248

	plants obtained using SDN 1, SDN 2 and ODM approaches		Regarding comment to lines 401-404, the GMO Panel considers the sentence already included in the document correct.	
VIB	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	There are no reasons to assume for ODM a similar possibility and/or frequency of off-target mutations as for SDN-1, SDN-2 and SDN-3, which all have a risk of generating fewer off-target mutations than conventional mutagenesis techniques. It would probably be reasonable to assume for ODM an even lower possibility and/or frequency of off-target mutations.	The GMO Panel thanks for the comment. The text of the opinion reflects the fact that very limited amount of information on the mechanisms and frequency of off-target effect for ODM technology is available in the literature. The sentence included in the document is considered by the GMO Panel the most conservative one.	249
Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG)	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	line 383 add after trabsgene - intragene or cisgene	The text has been amended accordingly.	250
GMO Office, National Institute of Public Health and the Environment (RIVM)	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	Line 407-410 EFSA concludes 'Indeed, in the absence of any transgene, the amount of experimental data needed for the risk assessment will mainly depend on the modified trait introduced and even less amount of experimental data would be needed for plants produced via SDN-1, SDN-2, and ODM compared to plants generated via SDN-3'. We agree with this statement, which seems rather obvious. It would be informative to indicate in the opinion (in general terms) which experimental data do not longer have to be supplied for genome edited plants.	The GMO Panel thanks for the comment. Depending on the methods which was used to generate the genome edited plant and the traits characterizing such products, the GMO panel may consider some data requirements not necessary for the risk assessment. For this reason, the "case-by-case" approach as described in the opinion on SDN3 is also applicable to genome edited plants. This position is in line with the conclusions of the opinion stating that the EFSA	251

			guidances are sufficient but can be only partially applied for the risk assessment of plants generated by the application of SDN1, SDN2, and ODM methods, especially when a transgene and/or exogenous DNA is not present in the final product.	
National Food Institute, Technical University of Denmark	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	This section could benefit from a direct comparison between the outcome of using SDN-1/SDN-2 and the outcome of using conventional mutagenesis. E.g. it is concluded that an even less amount of experimental data would be needed for SDN-1/SDN-2 compared to SDN-3. But what experimental data is really needed if compared with conventional mutagenesis?	The GMO Panel thanks for the comment. Depending on the methods which was used to generate the genome edited plant and the traits characterizing such products, the GMO panel may consider some data requirements not necessary for the risk assessment. For this reason, the "case-by-case" approach as described in the opinion on SDN-3 is also applicable to genome edited plants. This position is in line with the conclusions of the opinion stating that the EFSA guidances are sufficient but can be only partially applied for the risk assessment of plants generated by the application of SDN-1, SDN-2, and ODM methods, especially when a transgene and/or exogenous DNA is not present in the final product.	252
Plantum - Netherlands seed association	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	Lines 397-410 The various EFSA opinions are very well compared in a sequence from transgenics - via SDN-3 - to SDN-1 and 2, where increasingly fewer components can subsequently be applied and less data are needed for the risk assessment. This section does not refer to the amount of data – if any – that may remain after these subtractions. It would be helpful to know the opinion of the panel here.	The GMO Panel thanks for the comment. Depending on the methods which was used to generate the genome edited plant and the traits characterizing such products, the GMO panel may consider some data requirements not necessary for the risk assessment. For this reason, the "case-by-case" approach as described in the opinion on SDN-3 is also applicable to genome edited plants. This position is in line with the conclusions of the opinion stating that the EFSA	253

			guidances are sufficient but can be only partially applied for the risk assessment of plants generated by the application of SDN-1, SDN-2, and ODM methods, especially when a transgene and/or exogenous DNA is not present in the final product.	
French agency for Food, Environmental and Occupational Health & Safety (Anses)	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	Page 11, lines 384-387: "The EFSA opinion on SDN-3 concluded that the application of SDN-3 can induce off-target mutations but these would be fewer than those occurring with most mutagenesis techniques. Where they do occur, these changes would be the same types as those derived by 386 conventional breeding techniques (EFSA GMO Panel, 2012a)." : same comment as on lines 89-92 and 340-345. Page 11, lines 389-392: "In case of ODM, although very limited amount of information on the mechanisms and frequency of off-target effect is available in the literature, it is reasonable to assume that the same conclusions also apply since this technology is based on sequence-specific site recognition as for SDN-based methods.": this sentence has to be rephrased, because the lack of information available in the literature does not mean that off-target effect does not exist or that its occurrence is very low. The same comment applies to lines 350-352. Page 11, lines 393-396: "The conclusion addressing the risk assessment of the introduced transgene is not applicable because of the reason outlined in point 1. However, the GMO Panel considers that in some cases the SDN module could be stably introduced as a transgene in the plant genome. In these cases, the obtained plant should be considered a transgenic plant.": this could also be the case when the modified allele and associated trait present in the final product have never been described before (see comment on lines 300-301).	Regarding comment to lines 384-387, please refer to the responses provided for comment to lines 89-92 and 340-345. Regarding comment to lines 389-392, the text of the opinion has been modified to reflect the fact that very limited amount of information on the mechanisms and frequency of off-target effect for ODM technology is available in the literature. Regarding comment to lines 393-396, the GMO Panel takes note of the comment. Depending on the methods which was used to generate the genome edited plant and the traits characterizing such products, the GMO panel may consider some data requirements not necessary for the risk assessment. For this reason, the "case-by-case" approach as described in the opinion on SDN-3 is also applicable to genome edited plants. This position is in line with the conclusions of the opinion stating that the EFSA guidances are sufficient but can be only partially applied for the risk assessment of plants generated by the application of SDN-1, SDN-2, and ODM methods, especially when a transgene and/or exogenous DNA is not present in the final product.	254

Corteva Agriscience	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	We welcome the clear conclusions that the EFSA GMO Panel reached. In addition to the comments made to the previous sections, we would like to raise the issue of the combining the approach of single first assessment and risk assessment of all stacked events. As companies ceased to submit applications for cultivation of stacked transgenic events, it might have become less clear to EU regulators that in many cases combining of different mode of actions (e.g., for control of pests and pathogens) should be encouraged and sometimes even required to delay resistance formation to pests and pathogens. For crops produced using SDN technologies it might also be preferred by the developer to combine multiple adjusted disease resistance alleles in one or more steps and this should be encouraged by the regulatory framework as it is in the benefit to all that resistance formation of pests and pathogens is delayed. Similarly, one or more beneficial and independent mutations might be combined, as has been done safely since the start of agriculture, and for which no credible hypothesis of hazard could be identified. As such, stacks are not different from those where mutations are combined through conventional breeding. Therefore, we ask for flexibility and that the approach of risk assessment of single first and after that risk assessment for breeding stacks is removed for crops produced using SDN-1, SDN-2, and ODM genome editing. A confirmation in the product of interest (whether single or stack) that it does not contain unintentionally inserted foreign DNA and is therefore as safe as conventionally bred plants should be sufficient and there should only be a need for additional assessment in case credible risks are defined by the problem formulation.	The GMO Panel thanks Corteva and takes note of the comment related to the operational definition of single and stacked events when referring to genome edited plants.	255
European Plant Science Organisation, EPSO	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	Line 408-410: EPSO agrees with the EFSA opinion that in the absence of transgenes less data would be needed for risk assessment of plants produced via SDN-1, SDN-2, and ODM as compared to plants generated via SDN-3.	The GMO Panel thanks EPSO for the comment.	256
Haut Conseil des biotechnologies (High Council for Biotechnology)	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2	l. 386. As for l. 174, "the same types" need to be clarified. l. 384-392 (Point 2). See the comments developed in 3.2.2.2.2. l. 393-395. As commented in 3.2.2.2.2, plants containing an active SDN module should not just be considered as standard transgenic plants. Further analysis should be required considering the system is active and may generate further modifications during the deliberate release, or following hybridization with, or crossing into, another genotype. Furthermore, the point developed in l. 364-367 in case the applicant claims that the final product has not retained any inserted DNA should be stated in this section.	Regarding comment to line 386, please refer to the GMO Panel response to comment to line 174. Regarding comment to lines 384-392, please refer to the GMO Panel responses related to section 3.2.2.2.2. Regarding comment to line 393-395, the text has been modified to	257

	and ODM approaches	l. 401-404. "the two EFSA guidances are sufficient but can be only partially applied for the risk assessment of plants generated by the application of SDN-1, SDN-2, and ODM methods. Indeed, those requirements related to the presence of transgenes are not relevant because of the reason outlined in point 1." The wording "can be only partially" is confusing. Furthermore, "sufficient" is incorrect since additional requirements have been identified. We suggest instead: "the two EFSA guidances are partly applicable for the risk assessment of plants generated by the application of SDN-1, SDN-2, and ODM methods. Requirements related to the insertion of transgenes may not be needed while others may be added regarding assessment of the absence of any transgene or any DNA sequence potentially derived from the methods used to generate the intended modification, assessment of off-target effects, and further analysis in case an active SDN module is still present in the final product." l. 402-404. Make sure to clarify that the requirements related to the presence of transgenes are still relevant in case of stable integration of the SDN module in the plant genome (see point 3). l. 409-410. "Indeed, in the absence of any transgene, the amount of experimental data needed for the risk assessment will mainly depend on the modified trait and even less amount of experimental data would be needed for plants produced via SDN-1, SDN-2, and ODM compared to plants generated via SDN-3." This seems to contradict what was mentioned earlier in paragraph 3.2.2.1 regarding the sequence of the modified allele, which, in one extreme scenario, may be present in a consumed variety of the same species, or, in the other extreme scenario, may have never been described before, or in intermediate scenarios, may be found in other species, sexually compatible or not. Depending on the cases, various amounts of experimental data may be required for the risk assessment. This is not related to the presence of a transgene: whether the final sequence is the result of a modification (for SDN-1, -2 and ODM) or a full insertion (for SDN-3), the amount of experimental data will still depend on the novelty of the sequence, not just on the modified trait. A novel sequence may lead to an altered, but not novel, trait. It seems that less amount of data should be required for an already described sequence, irrespective of the way it was generated. The only difference for SDN-1, -2 and ODM compared to SDN-3 should be that there is no need for assessment of the impact of the physical insertion of the transgene in a new locus in the genome. Similarly, regarding data related to the modified trait, the amount of experimental data will vary according to the novelty of the trait, whether the plants were produced via SDN-1, SDN-2, ODM, or SDN-3. Equal amounts of experimental data in relation to the modified trait may be required for plants produced via SDN-1, SDN-2, ODM and SDN-3, these amounts being equally more important for novel traits. Finally, we regret that the issue of multiplexed targeted mutagenesis has not been considered in the document. Could EFSA address the possibility of multiplexing using these techniques and its consequences on the corresponding risk assessment?	better clarify that the presence of the transgene will be risk assessed according to all the requirements laid down in the EU GMO regulation. Regarding comment to lines 401-404, the GMO Panel considers the EFSA guidelines sufficient meaning that no new requirements have been identified for the risk assessment of genome edited plants generated by the application of SDN-1, SDN-2, and ODM methods. Regarding the comment to line 402-404, the GMO Panel considers the section clear enough. It should be noted that the all the considerations related to the presence of a transgene and its risk assessment have been described in section 3.2.2.2.2 and point 3 of this section. Regarding comment to line 409-410, the GMO Panel considers that what stated in point 4 agrees with the content of paragraph 3.2.2.1. Regarding the multiplexing approach, the GMO Panel understands that the term "multiplexing" used in the comment may refer to the simultaneous mutation of multiple plant genomic loci. Although multiplexing approach is not specifically discussed in the opinion, the GMO Panel considers that all the considerations included in the opinion on SDN-based methods are also applicable to multiplexing approaches. Moreover, it should also be noted that multiplexing is not specific to	

			SDN/ODM approaches as it can also be achieved by transgenic and conventional breeding approaches. The GMO Panel would also like to remind that the "case-by-case" approach can also be applied to genome edited plants. The GMO Panel knows that a complexity of scenarios is possible due to the application of SDN-based methods. In this regard, the GMO Panel refers to the mandate on GM plant generated via synthetic biology approaches.	
Testbiotech	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	[Line 380-410, delete and replace, first part, see also below:] "The criteria applied by EFSA (2012a) to distinguish conventional methods of breeding from transgenesis and genome editing methods need to be re-considered. EFSA (2012a) defines conventional breeding "as methods used by plant breeders for the improvement of commercial varieties and where the resulting plants/varieties are not covered by the legal definitions of genetic modification (Directive 2001/18/EC)." Scientifically, this definition is without any substance because it is not based on any scientific criteria. Further, in regard to regulation it is questionable, since there is an ongoing debate on regulation for processes of random mutagenesis, which so far was thought to be non-regulated (Conseil d'État, 2020). Therefore, it is suggested to use a definition which is based on scientific criteria. Such a definition is necessary to avoid any mixing of regulatory decision-making, which is in the remit of the risk manager and the legislator, with the issues of regulatory science which are within the remit of EFSA. More specifically, it is needed to make comparisons between the respective methods and also to come to reliable conclusions. The following criteria should be applied to differentiate between old and new genome techniques on the one hand, and methods used in conventional breeding on the other hand: a) In the case of conventional breeding, the first step requires a high degree of genetic diversity that subsequently provides the basis for further crossing and selection. To increase the genetic diversity, non-targeted mutagenesis can be applied by using chemical or physical effectors. In this case, the resulting genomic changes are intended as they increase the genetic diversity. In this context, it should be noted that due to the methods used in conventional breeding, some genetic alterations are more frequently observed than others. Inherent natural inheritance mechanisms such as the distance between two genes on a chromosome, recombinatorial hot spots, gene clusters, large genomes, linkage drag, cellular repair mechanisms and epigenetic effects allow some changes and gene combinations to occur more frequently than others, while some have to be considered as unlikely or even very unlikely. b) The situation in regard to SDN-1 and SDN-2 applications as well as other methods of genetic	Regarding the comment for lines 380-410, the EFSA scientific opinion on SDN-3 was developed by comparing the type of outcome and mutations produced by SDN-3 to those generated by conventional breeding, including random mutagenesis. For this reason, the GMO Panel followed the same approach for SDN-1, SDN-2, and ODM, in order to be able to assess the applicability of section 4 and conclusions of the opinion on SDN-3 to plant developed via these approaches. The GMO Panel was not mandated to provide a new definition of "conventional breeding". Please note that the footnote 5, which reported the definition as stated in the comment, has been removed and replaced by the list of techniques relevant for a comparison as indicated in the opinion on SDN-3 (section 3.2.1).	258

		engineering is very different: (1) these applications are typically not meant to increase genetic diversity in a non-targeted way. Therefore, unintended changes in the genome have to be seen as undesirable effects; (2) Tools such as CRISPR/Cas make a much larger part of the genome available for genetic changes compared to conventional breeding; it allows biological characteristics to be generated that were previously not achievable; (3) Genetic engineering uses biological mutagens (molecules) as effectors which are intended to target biological mechanisms in the genome or in gene regulation. Whatever the case, there are substantial and even fundamental differences between methods of genetic engineering and conventional methods of breeding. The EFSA (2012a) failed to address these differences properly and therefore the conclusions in this previous document are not sufficiently valid. For example, the finding that SDN-3 would result in less off-target effects might be appropriate if compared with previous methods of transgenesis. Such a finding would still need further investigations in regard to unintended effects caused by the SDN application (see above). However, in the end, one might be able to come to a reliable conclusion. Nevertheless, there is no major scientific benefit if SDN methods are compared to conventional plant breeding methods meant to enhance genetic diversity. The most relevant conclusion in this case would be that the differences between methods, goals and results are greater than the similarities. In summary, the applicability of the conclusions in the EFSA opinion on SDN-3 in regard to plants obtained using SDN-1, SDN-2 is very limited and not sufficient to guide risk assessment: 1. Given the findings above: (i) several steps during the technical processes, (ii) new combinations of genetic information and the resulting unintended and intended biological characteristics, as well as (iii) on-target and off-target effects caused by the activities of the biological mutagens, may pose substantial challenges for risk assessment. The set of data needed for risk assessment of SDN-1 and SDN-2 (and potentially ODM applications) might in some cases be similar to those requested for SDN-3. However, in many cases it might substantially deviate, with even more data being requested. [Text is continued in next chapter]		
Umweltbundesamt (Environment Agency Austria) on behalf of the Austrian lead Competent Authority, the Federal Ministry of Social Affairs, Health, Care and Consumer Protection.	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	Lines 384ff: As indicated in response to previous sections we find this conclusions too generalized. The draft opinion assumes that SDN-1 SDN-2 and ODM methods can induce off-target mutations but these would be fewer than those occurring with most mutagenesis techniques. This statement omits that the frequency of induced mutations is intentionally quite different in different approaches implemented in conventional breeding. It also disregards that a stringent selection and back-crossing regime is typically applied in such breeding programmes with the aim to identify and remove unwanted mutations. Similar scrutiny has to be employed with SDN-1 SDN-2 and ODM methods and the results need to be confirmed during risk assessment. The draft opinion also concludes that if off-target mutations occur, "these changes would be the same types as those derived by conventional breeding techniques (EFSA GMO Panel, 2012a)". As discussed with regard to previous sections the pattern of off-target mutations induced by SDN-applications may be significantly different from either spontaneous or induced mutations as regards frequency, genomic distribution, type of changes and outcomes. This needs to be taken into account	To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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	259

		appropriately. The conclusion offered for ODM is an assumption at best with a view to the indicated significant mechanistic uncertainty concerning this technology. This needs to be stated correctly. Line 409f: We note that the amount of experimental data that would be need-ed for assessment of the introduced traits depends on the novelty of the created traits and therefore it cannot be assumed that in general the necessary amount of experimental data would less compared to plants generated via SDN-3. The draft opinion has to be revised accordingly.	random mutagenesis. In order to clarify its positions, the GMO Panel has revised the text of the opinion of section 3.2 accordingly, including some additional relevant references. Regarding the conclusion on ODM, the text has been revised and considered adequate. Regarding comment to line 409, the GMO Panel considers that the conclusion in the EFSA opinion on SDN-3 on the "case-by-case" approach still applies to plants developed via SDN-1, SDN-2, and ODM methods. This implies that not always the amount of data needed for the risk assessment would be less since this will depend on the type of product under assessment.	
V, Ganesh kumar	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	In Line number 408 to 410 of Page 11 to 12 it was mentioned that "the amount of experimental data needed for the risk assessment will mainly depend on the modified trait". It is not clear, it needs to be stated with more details on types of experimental data required, and it will be highly useful if the data required for different possible scenarios is included here. If the details are not given, it will lead to uncertainty for the product developer (who will be in a position to know details only upon the case by case analysis of the developed product, but not during the product conception stage) and will hugely impact the cost and pace of the product development process. Also, the data requirement should be based on the type of sequence modification and should not be mentioned as the developed trait (as it is technically not logical).	The GMO Panel was not mandated to provide a complete list of studies which are deemed necessary or not for the risk assessment of genome edited plants. Please note that this document is not meant to replace the current requirements under IR 503/2013 and EFSA guidances which still applies for the risk assessment of genome edited plants. Indeed, the EUCJ Case C-528/16 on mutagenesis has clarified that Directive 2001/18/EC is applicable to genetically modified organisms (GMOs) obtained by mutagenesis techniques like SDN-based methods.	260
Sciensano	3.3 ToR2 of the mandate: Applicability of the Conclusions of	Line 387: "As SDN-1 and SDN-2 techniques use the same molecular mechanisms to generate DSB as SDN-3", this sentence should include the same nuance as described at line 314 to take into account base editing and prime editing approaches.	The text refers to the EFSA opinion on SDN-3, a method that requires DSB in order to insert exogenous DNA. The conclusions on off-targets included in the	261

	the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches		opinion on SDN-3 are applicable to those SDN-1 and SDN-2 methods which can induce DSB.	
Cornell University's Alliance for Science	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	• The Panel accurately determined that the existing Guidances for food and feed (EFSA GMO Panel, 2011) and environmental risk assessment (EFSA GMO Panel, 2010) should only be partially applied for the risk assessment of plants generated via SDN-1, SDN-2 and ODM techniques. The Panel correctly distinguished that the two Guidance's requirements related to the presence of transgenes are not relevant in cases where these techniques produced plants that do not have exogenous DNA as a final product and that on a case-by-case basis lesser amounts of event-specific data are needed for the risk assessment process for those plants. These science-based conclusions that differentiate the risk assessment procedures that should be applied to the different products created through the use of SDN1, SDN2 and ODM techniques should be adopted by EFSA for its review of all SDN1, SDN2, and ODM plant products. However, The GMO Panel should provide further clarity and details on how EFSA will conduct the risk assessment of plants produced by SDN-1, SDN-2, and ODM methods for those that contain exogenous DNA and those that do not. • We would like the Panel to clarify and expressly state which portions of the risk assessment guidelines apply to SDN1, SDN2 and ODM without exogenous DNA; which tests and data and risk analysis from the guidances are applicable? • If very little of the two EFSAs guidances on GMOs would apply for risk assessments to plants generated by these techniques, there may be no scientific justification for applying them at all.	The GMO Panel thanks for the comment. Depending on the methods which was used to generate the genome edited plant and the traits characterizing such products, the GMO panel may consider some data requirements not necessary for the risk assessment. For this reason, the "case-by-case" approach as described in the opinion on SDN-3 is also applicable to genome edited plants. This position is in line with the conclusions of the opinion stating that the EFSA guidances are sufficient but can be only partially applied for the risk assessment of plants generated by the application of SDN-1, SDN-2, and ODM methods, especially when a transgene and/or exogenous DNA is not present in the final product.	262
Federal Agency for Nature Conservation	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	Lines 384-389: We do not share the draft's statement that the conclusions of EFSA (2012) that SDN-3 interventions induce fewer off-target mutations and of the same type as conventional breeding are applicable also to SDN-1 and SDN-2 interventions. This is for two main reasons: (i) it disregards the potential and the possibilities of SDN-1 and SDN-2 of multiplexing and of deep genomic interventions (see comment under 3.1.1) and (ii) it disregards that conventional breeding and genome editing take two distinct approaches to achieve a new trait (see comment under 2.1.3).	The GMO Panel understands that the term "multiplexing" used in the comment may refer to the simultaneous mutation of multiple plant genomic loci. Although multiplexing approach is not specifically discussed in the opinion, the GMO Panel considers that all the considerations included in the opinion on SDN-based methods are also applicable to multiplexing approaches. Moreover, it should also be noted that multiplexing is not specific to SDN/ODM approaches as it can	263

			also be achieved by transgenic and conventional breeding approaches. The GMO Panel would also like to remind that the "case-by-case" approach can also be applied to genome edited plants. The GMO Panel knows that a complexity of scenarios is possible due to the application of SDN-based methods. In this regard, the GMO Panel refers to the mandate on GM plant generated via synthetic biology approaches. The GMO Panel also refers the contributor to the responses given for the comments in section 2.1.3.	
Envirnonmental association Za Zemiata	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	[Line 380-410, delete and replace:] "The criteria applied by EFSA (2012a) to distinguish conventional methods of breeding from transgenesis and genome editing methods need to be re-considered. EFSA (2012a) defines conventional breeding "as methods used by plant breeders for the improvement of commercial varieties and where the resulting plants/varieties are not covered by the legal definitions of genetic modification (Directive 2001/18/EC)." Scientifically, this definition is without any substance because it is not based on any scientific criteria. Further, in regard to regulation it is questionable, since there is an ongoing debate on regulation for processes of random mutagenesis, which so far was thought to be non-regulated (Conseil d'État, 2020). Therefore, it is suggested to use a definition which is based on scientific criteria. Such a definition is necessary to avoid any mixing of regulatory decision-making, which is in the remit of the risk manager and the legislator, with the issues of regulatory science which are within the remit of EFSA. More specifically, it is needed to make comparisons between the respective methods and also to come to reliable conclusions. The following criteria should be applied to differentiate between old and new genome techniques on the one hand, and methods used in conventional breeding on the other hand: a) In the case of conventional breeding, the first step requires a high degree of genetic diversity that subsequently provides the basis for further crossing and selection. To increase the genetic diversity, non-targeted mutagenesis can be applied by using chemical or physical effectors. In this case, the resulting genomic changes are intended as they increase the genetic diversity. In this context, it should be noted that due to the methods used in conventional breeding, some genetic alterations are more frequently observed than others. Inherent natural inheritance mechanisms such as the distance between two genes on a chromosome, recombinatorial hot spots, gene clusters, large genomes, linkage drag, cellular repair mechanisms and epigenetic effects allow some changes and gene combinations to occur more frequently than others, while some have to be considered as unlikely or even very unlikely.	Regarding the comment for lines 380-410, the EFSA scientific opinion on SDN-3 was developed by comparing the type of outcome and mutations produced by SDN-3 to those generated by conventional breeding, including random mutagenesis. For this reason, the GMO Panel followed the same approach for SDN-1, SDN-2, and ODM, in order to be able to assess the applicability of section 4 and conclusions of the opinion on SDN-3 to plant developed via these approaches. The GMO Panel was not mandated to provide a new definition of "conventional breeding". Please note that the footnote 5, which reported the definition as stated in the comment, has been removed and replaced by the list of techniques relevant for a comparison as indicated in the opinion on SDN 3.	264

		b) The situation in regard to SDN-1 and SDN-2 applications as well as other methods of genetic engineering is very different: (1) these applications are typically not meant to increase genetic diversity in a non-targeted way. Therefore, unintended changes in the genome have to be seen as undesirable effects; (2) Tools such as CRISPR/Cas make a much larger part of the genome available for genetic changes compared to conventional breeding; it allows biological characteristics to be generated that were previously not achievable; (3) Genetic engineering uses biological mutagens (molecules) as effectors which are intended to target biological mechanisms in the genome or in gene regulation. Whatever the case, there are substantial and even fundamental differences between methods of genetic engineering and conventional methods of breeding. The EFSA (2012a) failed to address these differences properly and therefore the conclusions in this previous document are not sufficiently valid. For example, the finding that SDN-3 would result in less off-target effects might be appropriate if compared with previous methods of transgenesis. Such a finding would still need further investigations in regard to unintended effects caused by the SDN application (see above). However, in the end, one might be able to come to a reliable conclusion. Nevertheless, there is no major scientific benefit if SDN methods are compared to conventional plant breeding methods meant to enhance genetic diversity. The most relevant conclusion in this case would be that the differences between methods, goals and results are greater than the similarities. In summary, the applicability of the conclusions in the EFSA opinion on SDN-3 in regard to plants obtained using SDN-1, SDN-2 is very limited and not sufficient to guide risk assessment.		
BUND e.V. / Friends of the Earth Germany	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	Line 383 ADD sentence after "transgene.": "Still, it must be taken into account whether fragments of foreign DNA remain unintentionally (Jupe et al. 2019, Andersson et al. 2018, Michno et al. 2020), this must be part of any risk assessment." Line 403 REPLACE "not" with: "only in parts" Line 404 ADD after "point 1" "; it is applicable though in the case of the reason outlined in point 3." Line 407-410 DELETE sentence "Indeed .. SDN-3." and REPLACE with new sentences: "Although in case the absence of any transgene sequence has been truly shown some experimental data may not be needed for the risk assessment, there are specific risks attached to the use of SDN-1 and SDN-2. These include large deletions, insertions, rearrangements, exon skipping and formation of new mRNA molecules and proteins with potentially new functions (Kapahnke et al. 2016, Lalonde et al. 2017, Mou et al. 2017, Smits et al. 2019, Skryabin et al. 2020). Regulatory sequences may also be altered leading to proteins produced in other tissues and at other developmental stages exerting influence on the interaction of the GM plants with their einvironment. These potential unintended	The GMO Panel considers that for the comments related to lines 383, 403, 404, and 407-410 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.	265

		alterations have not been addressed so far and have not been taken into consideration in the 2012-opinion. They, therefore, require experimental data and specific risk assessment."		
GenØk Centre for Biosafety	3.3 ToR2 of the mandate: Applicability of the Conclusions of the EFSA opinion on SDN 3 to plants obtained using SDN 1, SDN 2 and ODM approaches	**Copied from the submitted pdf file:** Conclusions do not reflect the current scientific knowledge on the safety of such techniques Throughout this document we have shown how the analysis by EFSA was narrow and limited to the assessment of the presence of transgenes at the intended site of modification. With the aim of not being repetitive, we believe that these conclusions and the conclusions under section #4 are not legitimate to the current scientific knowledge presented in this review and we urge that the Panel revises its conclusions according to the review and comments made in this document.	The GMO Panel takes note of the comment. Each comment was taking into consideration while reviewing the text of the document. Changes to the text were introduced whenever considered necessary.	266
ENSSER	4. Conclusions	Concerning ToR1 and ToR2: Given the severe shortcomings of this draft opinion and its failure to provide a whole picture as well as an up-to date picture (esp. for SDN-3), we cannot concurr with this conclusion. According to our analysis there are new hazards and new risks arising from the application of SDN-1, SDN-2 and ODM approaches, in particular with the ability to achieve complex and deep changes in the genome and metabolic pathways with serial or multiplexing applications of SDN-1 and/or -2. Equally important is the spectrum of unintended effects and unintended modifications, including on-target unintended effects, where one knock-out may be sufficient to give rise to a new RNA or protein or a shift in compound composition. Chosing the right baseline and comparator to fully ensure environmental and human and animal health safety is crucial, even more as we are facing severe biodiversity loss and the collapse of ecosystems. Please consider all our contributions to all the different sections of this draft opinion and please take the time for a thorough assesmment and evidence-based evaluation of this complex and important issue. We are looking forward to a new draft opinion. We thank you for your efforts.	The GMO Panel thanks ENSSER and the effort made by the contributor in providing valuable comments to the opinion. The GMO Panel has taken into consideration all the comments and when necessary the text of the opinion has been modified accordingly.	267
EuropaBio	4. Conclusions	Lines 416-417: EuropaBio agree with the conclusion of the EFSA GMO Panel that plants developed using SDN-1, SDN-2 and ODM approaches are unlikely to represent any additional hazards compared with SDN-3 and conventional breeding techniques, including mutagenesis. The principle of proportionality, especially in view of similar and safe products resulting from conventional breeding should guide the need for data to enable risk assessment. Lines 419-425: EuropaBio suggests that EFSA discussed here that using the case-by-case approach and the application of problem formulation is useful in guiding the assessment to establish what data requirements outlined in previous guidance are relevant for the EFSA risk assessment. For data requirements not relevant for a particular product the derogation clause in IR 503/2013 should be used. We also suggest that, EFSA considers discussions with applicants prior and during submission to ensure that fit-for-purpose risk assessments are produced.	The GMO Panel thanks EuropaBio and takes note of the comments.	268
Logos Environmental	4. Conclusions	As a general comment, this draft Opinion appears to seek to minimise recognition of any errors created by genome editing. It does not do EFSA credit. Errors such as exon skipping and off target effects require rigorous assessment if genome-edited crops are to be considered for use in	The GMO Panel takes note of the comment. In developing this opinion, the GMO Panel considered	269

		agriculture. The Opinion appears to focus on the most recent techniques such as "DNA-free" genome editing, when the reality is that those created by insertion of transgenes coding for genome editing components are more likely to be the subject of applications for deliberate release and marketing in the first instance.	the implications of the application of genome editing techniques (SDN-1, SDN-2, and ODM) in plants for their risk assessment. Please, refer to section 3.2 of the opinion and the related comments and responses provided by the GMO Panel on the aspects raised in this comment.	
Institute of experimental Botany, Czech Academy of Science	4. Conclusions	Again we agree with the EFSA conclusions that the guidelines for risk analysis of SDN-3 derived plants are only partially applicable to the safety assessment of plants derived using SDN-1 and/or SDN-2 techniques, since most of the assessment is oriented on the analysis of inserted foreign DNA that is not present in these latter events. We believe that the principle of proportionality should be applied in the risk assessment and that the risk assessment should follow a similar pathway as risk assessment of plants derived using conventional mutagenesis focusing on traits and the hazards that these may pose.	The GMO Panel thanks for the comment.	270
Norwegian Scientific Committee for Food and Environment (VKM)	4. Conclusions	In line 415 it is stated: "these plants will not present any of the potential hazards related to the insertion of a transgene". To us, it is not completely clear whether this relates to hazards caused by insertion process itself, or to hazards caused by the inserted sequence. Please clarify.	In order to better clarify the sentence, the text of conclusion was modified accordingly.	271
Euroseeds	4. Conclusions	Line 415-418: Again: The conclusions justify elaborating on the principle of proportionality and non-discrimination specifically in view of like- and safe products resulting from conventional breeding practices as outlined above. The breeders selection process as well as the official trialling process for all plant varieties independent of the breeding technology used generally occurs over multiple geographies and multiple years in order to observe and exclude potential variability, keeping only those varieties that will meet consumer and grower expectations and show reliable performance under different environmental conditions. Independent of the breeding technology used, potentially commercial varieties are tested for: • Geographic and agricultural/horticultural production system adaptation • Performance characteristics, relative to existing commercial varieties • Processing characteristics appropriate for that crop, such as milling for wheat, sugar yield for sugar beets; oil quality for canola and sunflower; or storage characteristics for fruits and vegetables • End-user characteristics (as appropriate for that crop), such as protein content or bread-making characteristics for wheat, oil quality for oilseed rape or flavour characteristics for vegetables and fruits • Regardless of the tools used for breeding, the goal is always the same: To first create genetic diversity in a population of plants and through multiple years of field trials and testing develop new plant varieties that reliably produce safe, nutritious, good tasting food (15) .	The GMO Panel thanks Euroseeds and takes note of the comment.	272

		All partners of the agricultural production chain take their individual responsibilities to contribute to an environmentally safe production of high quality feed & food. Responsibility for product safety is always linked to the specific product developed for marketing. Breeders may thus be considered responsible for varieties with "safe" genetics intended for feed & food production. The comprehensive regulatory framework for EU-breeders, seed producers, processors et.al. is already in place (16). (15) https://www.euroseeds.eu/app/uploads/2020/03/PlantBreeding_as_part_of_the_Breeding_Cycle.png (16) FRom farm to fork: the regulatory status of non-GMO plant innovations under current EU law https://www.altius.com/images/Publications/De%20Jong/ARTICLE_-_de_Jong_et_al._-_From_farm_to_fork_BSLR_2018.pdf		
SETA (Science and Technology in Agriculture)	4. Conclusions	Lines 422-425 As SDN-1, SDN-2 and ODM aim at mutagenize resident DNA sequence(s), a number of requirements of the existing guidance's that are linked to the presence of a transgene are not relevant for the assessment of SDN-1, SDN-2 and ODM plants as the final product does not contain a DNA sequence or "genetic material (that) has been altered in a way that does not occur naturally (by mutagenesis,) by mating and/or natural recombination" (https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2001L0018:20080321:EN:PDF). SETA suggests that SDN-1, SDN-2 and ODM are mutagenesis, more precise and less unpredictable than the mutations that are even excluded from the anachronistic 2001/18 Directive.	The GMO Panel thanks SETA and takes note of the comment.	273
Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG)	4. Conclusions	WGG supports the conclusions.	The GMO Panel thanks WGG for the comment.	274
BIOTRIN, z.s.	4. Conclusions	We share the same opinion as EFSA, that there are no new hazards expected, which would be associated with plants produced by SDN-1, SDN-2 and/or ODM techniques when compared to SDN-3 techniques or conventional breeding. We are of the opinion, that the risk assessment for SDN-1, SDN-2 and/or ODM techniques should be proportionate and should not pose any further unnecessary burden for applicants. Mainly in the case, where there is no exogenous DNA in the final product. Case-by-case approach should be preferred to ensure fit-for-purpose risk assessments. Based on this, the risk assessment for SDN-3 techniques seems to be only partially applicable for SDN-1, SDN-2 and/or ODM techniques. Instead of this, it should be more analogous to principles applied when similar plants are derived through conventional breeding, with the accent on final traits and the possible risks.	The GMO Panel thanks BIOTRIN for the comment.	275
Union Française des Semenciers	4. Conclusions	-Line 415-418: Again: The conclusions justify elaborating on the principle of proportionality and non-discrimination specifically in view of like- and safe products resulting from conventional breeding practices as outlined above. The breeding process is followed by field screening and official trialing process for registration over multiple geographies and multiple years. They allow to observe and exclude	The GMO Panel thanks Union Française des Semenciers and takes note of the comment.	276

		potential detriment, keeping only those varieties that will meet consumer and grower expectations and show reliable performance under different environmental conditions and farming practices. Independent of the breeding technology used, potentially commercial varieties are tested for: • Geographic and agricultural/horticultural production system adaptation, including abiotic stresses (drought, salted soils) and resistance to pests and diseases • Performance characteristics, compared to existing commercial varieties • Processing characteristics appropriate for that crop, such as milling for wheat, sugar yield for sugar beets; oil quality for canola and sunflower; or storage characteristics for fruits and vegetables • End-user characteristics (as appropriate for that crop), such as protein content or bread- making characteristics for wheat, oil quality for oilseed rape or flavour characteristics for vegetables and fruits • Regardless of the tools used for breeding, the goal is always the same: To first create genetic diversity in a population of plants and develop new plant varieties that reliably produce safe, nutritious, good tasting food (4) through multiple years of field trials, observations, analyses and testing. All partners of the agricultural production chain take their individual responsibilities to contribute to an environmentally safe production of high quality feed & food. Responsibility for product safety is always related to the specific product developed for marketing. The development of varieties with "safe" genetics intended for feed & food production is under the breeders' responsibility. The comprehensive regulatory framework for EU-breeders, seed producers, processors and so forth is already in place (5). (4) https://www.euroseeds.eu/app/uploads/2020/03/PlantBreeding_as_part_of_the_Breeding_Cycle.png (5) From farm to fork: the regulatory status of non-GMO plant innovations under current EU law https://www.altius.com/images/Publications/De%20Jong/ARTICLE_-_de_Jong_et_al._-_From_farm_to_fork_BSLR_2018.pdf		
Plant Biotechnology Society	4. Conclusions	see attached file	The GMO Panel took note of the comment.	277
Scientific Committee for GM food and Feed, Advisory Body, Czech Republic	4. Conclusions	It is concuded that "In relation to ToR2, the GMO Panel concludes that the existing Guidances for food and feed (EFSA GMO Panel, 2011) and environmental risk assessment (EFSA GMO Panel, 2010) are sufficient but can be only partially applied for the risk assessment of plants generated via SDN-1, SDN-2, and ODM approaches." We suggest to consider much bolder and accommodating wording (conditioned by the absence of foreign DNA in the final product), e.g.: "In relation to ToR2, the GMO Panel concludes that the existing Guidances for food and feed (EFSA GMO Panel, 2011) and environmental risk assessment (EFSA GMO Panel, 2010) are more than sufficient, so only a subset of the requirements should be applied for the risk assessment of plants generated via SDN-1, SDN-2, and ODM approaches in case foreign DNA is not present in the final product."	The GMO Panel thanks for the comment. The GMO Panel considers the sentence in the conclusions to be sufficiently clear.	278
GMO Office, National Institute	4. Conclusions	EFSA separates genome edited plants in two categories: genome edited plants without exogenous DNA deployed during the process and genome edited plants with exogenous DNA deployed during	The GMO Panel thanks RIVM for the comment. The GMO Panel	279

of Public Health and the Environment (RIVM)		the process (intentionally and unintentionally). The second category should -according to EFSA- be assessed as transgenic plants. Both categories are described together throughout the text. The opinion could be improved by separating both categories in the text. This could be done by stating upfront that gene edited plants using SDN-1, -2 and ODM are assumed not to contain any exogenous DNA deployed during the process and that the current EFSA opinion is based on this assumption. In this way it can clearly be described in how far the SDN-3 opinion is applicable or not to gene edited plants and the kind of data that no longer has to be supplied. Thereafter a section could be included where is mentioned what would be the practical consequence for the risk assessment in case there is exogenous DNA present in the plant that is deployed during the process.	adhered to the terms of reference provided by the European Commission and formulated the conclusions accordingly. The aspects related to the risk assessment of the exogenous DNA that could be still present in the final product are described in section 3.2.2.2.2.	
European Coordination Via Campesina	4. Conclusions	As a general comment, this draft Opinion appears to seek to minimise recognition of any errors created by genome editing. Errors such as exon skipping and off target effects require rigorous assessment if genome-edited crops are to be considered for use in agriculture. The Opinion appears to focus on the most recent techniques such as "DNA-free" genome editing, when the reality is that those created by insertion of transgenes coding for genome editing components are more likely to be the subject of applications for deliberate release and marketing in the first instance. When assessing SDN1 and 2 (the same for other new GM techniques), we should also point out the risks of irreversible contamination of wild and cultivated plants, as well as of soil microorganisms, whether by intentionally or unintentionally modified genes, or by changes in the interactions between cultivated GMOs and the environment (monocultures, modification of trophic chains and microbial or soil fungus populations, increased use of herbicides, invasive plants, such as amaranth in the US). This is not only an economic problem, but also a biosecurity problem in terms of protecting biodiversity and ecosystem balances.	The GMO Panel takes note of the comment. Please, refer to section 3.2 of the opinion and the related comments and responses provided by the GMO Panel for the aspects raised in this comment. The environmental considerations highlighted in the comment apply also to plants obtained by the application of traditional transgenesis. In this respect, these aspects do not represent new hazards associated to plants produced via the application of SDN- and ODM-based methods. It should be noted that the environmental risk assessment (ERA) is a pillar of the risk assessment process for all GM plants.	280
National Food Institute, Technical University of Denmark	4. Conclusions	The conclusion includes the following statement: "…did not identify any additional hazard associated to the use of SDN-1, SDN-2 and ODM approaches …as compared to … conventional mutagenesis". Unfortunately, this conclusion does not seem to be based on a direct comparison with conventional mutagenesis, which is making the conclusion unclear. If no additional hazard are present, why should plant developed with SDN-1 or SDN-2 have to go through the GMO application procedure and not through the same procedure as plants developed with conventional mutagenesis? Could EFSA justify this? Or would EFSA prefer the same strict regulation for traditional bred plants as for GMP due to the uncertainties and risk that we can foresee? Without this kind of evaluations and comparison to traditional more or less risky traditional breeding techniques GMO will still be considered as the results of using high risk methods. As scientists with knowledge about GMO we should not contribute to this simplified hypothesis.	The GMO Panel thanks for the comment. The document was developed by strictly adhering to the terms of reference provided by the EC. As stated in the background information, a comparison between SDN-3 and conventional breeding was carried out in the EFSA opinion on SDN-3, in particular on off-target effect. For this reason, the GMO Panel considered necessary to explicit that no additional hazards have been identified for plants developed via SDN-1, SDN-2, and	281

			ODM approaches compared to both SDN-3 and conventional breeding. It should be noted that conventional breeding including mutagenesis is exempted from the Directive 2001/18/EC while the EUCJ case C-528/16 has clarified that Directive 2001/18/EC is applicable to genetically modified organisms (GMOs) obtained by mutagenesis techniques that have emerged since its adoption (i. e. SDN-based approaches). 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.	
German Plant Breeders' Association (BDP - Bundesverband Deutscher Pflanzenzuechter e.V.)	4. Conclusions	BDP agrees with the conclusions regarding applicability of previous EFSA opinions and existing guidance. However, also in the conclusions the principle of proportionality as outlined in our comments to the Terms of Reference should be considered and applied such that the Efsa opinion is put in a broader perspective. It should be highlighted that any measures need to be appropriate and non-discriminatory to achieve the overall objective of safety, especially when taking the outcome of SDN1/2 and ODM approaches into account particularly in comparison to conventional breeding.	The GMO Panel takes note of the comment. The opinion has been developed by strictly adhering to the terms of reference provided by the European Commission. In section 3.3, the GMO Panel concludes that the case-by-case as described in the EFSA opinion on SDN-3 remains valid also for the risk assessment of plants obtained using SDN-1, SDN-2, and ODM methods.	282
Plantum - Netherlands seed association	4. Conclusions	We refer to our remarks on the abstract. If components of the risk assessments (see remark on TOR2) are deemed relevant for SDN-1 and 2 products despite that fact that hazards additional to those occurring in conventional breeding are absent, then it will be very important to take into account proportionality between the data requirements in relation to the expected reduction of risk.	The GMO Panel takes note of the comment. The opinion has been developed by strictly adhering to the terms of reference provided by the European Commission. In section 3.3, the GMO Panel concludes that the case-by-case as described in the EFSA opinion on SDN-3 remains valid also for the risk assessment of plants obtained using SDN-1, SDN-2, and ODM methods.	283

COST Action CA18111 - Plant genome editing – a technology with transformative potential (PlantEd)	4. Conclusions	The conclusions justify elaborating on proportionality and non-discrimination in view of similar and generally safe products resulting from conventional breeding practices as outlined at multiple instances above.	The GMO Panel takes note of the comment.	284
French agency for Food, Environmental and Occupational Health & Safety (Anses)	4. Conclusions	Page 12, lines 412-425: This paragraph is incomplete, for the following reasons: 1) again, there is only mention of final products that do not contain any exogenous DNA. 2) the potential off-targets are not even cited. Additionally, Anses considers that the question of the off-targets has not been addressed properly.	The GMO Panel takes note of the comment. The opinion has been developed by strictly adhering to the terms of reference provided by the European Commission. The conclusions of the opinion should be read in conjunction to the content of both section 4 and conclusions of the EFSA opinion on SDN-3. In relation to point 2 raised in the comment, the GMO Panel refers the contributor to the section 3.2.2.2.2 of the opinion and the related comments and responses.	285
Corteva Agriscience	4. Conclusions	Lines 416-418: In light of the conclusion that no new hazards are "specifically linked to the genomic modifications produced via SDN-1, SDN-2 and ODM as compared to both SDN-3 and conventional breeding" (in bold by Corteva), we ask EFSA to confirm and additionally clarify that the purported "hazards" are no different than those from plants developed using techniques with a long history of safe use.	The GMO Panel thanks for the comment. The document was developed by strictly adhering to the terms of reference provided by the EC. As stated in the background information, a comparison between SDN-3 and conventional breeding was carried out in the EFSA opinion on SDN-3, in particular on off-target effect. For this reason, the GMO Panel considered necessary to explicit that no additional hazards have been identified for plants developed via SDN-1, SDN-2, and ODM approaches compared to both SDN-3 and conventional breeding. It should be noted that conventional breeding including mutagenesis is exempted from the Directive 2001/18/EC while the EUCJ case C-528/16 has clarified that Directive 2001/18/EC is applicable to genetically modified organisms (GMOs) obtained by	286

			mutagenesis techniques that have emerged since its adoption (i. e. SDN-based approaches). 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.	
Nature et Progrès Belgique	4. Conclusions	As a general comment, this draft opinion appears to seek to minimise recognition of any errors created by genome editing. Il does not do EFSA credit. Errors such as exon skipping and off target effects require rigorous assessment if genome-edited crops are to be considered for use in agriculture. The opinion appears to focus on the most recent techniques such as "DNA free" genome editing, when the reality is that those created by insertion of transgenes coding for genome editing components are more likely to be the subject of applications for deliberate release and marketing in the first instance.	The GMO Panel takes note of the comment. In developing this opinion, the GMO Panel considered the implications of the application of genome editing techniques (SDN-1, SDN-2, and ODM) in plants for their risk assessment. Please, refer to section 3.2 of the opinion and the related comments and responses provided by the GMO Panel on the aspects raised in the comment.	287
Haut Conseil des biotechnologies (High Council for Biotechnology)	4. Conclusions	l. 416. "Moreover, the GMO Panel did not identify any additional hazard associated to the use of the SDN-1, SDN-2 and ODM approaches as compared etc." Could the GMO Panel address the question of risk assessment of multiplexed modifications using these techniques? l. 419-425. See comment on line 401-404. Consistent with the overall analysis, we suggest: the two guidances "are partly applicable for the risk assessment of plants generated by the application of SDN-1, SDN-2, and ODM methods. Depending on the cases, requirements related to the insertion of transgenes may not be needed while others may be added regarding assessment of the absence of any transgene or any DNA sequence potentially derived from the methods used to generate the intended modification, assessment of off-target effects, further analysis in case an active SDN module is still present in the final product, and specific consideration regarding multiplexing where relevant."	Regarding the comment to line 416, the GMO Panel refers the contributor to the responses provided for comments on multiplexing modifications in section 3.2. Regarding the comment to lines 419-425, discussion on aspects like the assessment of absence of any exogenous DNA and the off-target effects can be found in section 3.2 of the opinion. Regarding the multiplexing approach, please refer to the responses provided for comments on multiplexing modifications in section 3.2.	288
Testbiotech	4. Conclusions	[Line 380-410, delete and replace, first part, see also above:] For example, if the newly generated gene combination results in profound changes of the plant metabolism, the comparative risk assessment may be challenged to an extent that goes far beyond the existing experience with transgenic plants or future SDN-3 applications.	The GMO Panel considers that for the comments related to lines 380-410 and 412-425, an explanation of the rationale for the proposed change is insufficient. Therefore,	289

		2. Also in regard to environmental risk assessment, there are new challenges that may go far beyond current experience with transgenic plants or considerations regarding SDN-3. These include changes in the composition of plants that may impact the food web, changes in the composition of plants that may impact plant communication and interaction with the environment, changes in the biological characteristics of the plants in regard to their invasiveness and next generation effects of plants with the potential to persist and propagate in the environment (see Testbiotech, 2020). Since these issues were not considered in the EFSA (2012a), also the assumptions and conclusions made in this previous document cannot be considered to be valid or sufficient in regard to the uses of SDN-1 and SDN-2. Since in regard to ODM, most relevant data are missing, no conclusion can be derived. 4 Conclusions [line 412-425 delete and replace:] "In relation to ToR1, the GMO Panel concludes that the assessment methodology presented in section 4 of the EFSA opinion on SDN-3 is partially applicable to SDN-1, SDN-2, and ODM. However, EFSA (2012a) did not provide a scientific basis for conventional plant breeding (using non-targeted chemical or physical mutagens) being compared to methods of genetic engineering using targeted biological mutagens. Therefore, EFSA (2012a) methodology has fundamental deficiencies. Beyond that, EFSA (2012a) did not consider the potential of SDN-1 and SDN-2 applications to penetrate the genome and cause profound alterations in the biological characteristics of plants without introducing any additional DNA sequences. As shown, risk assessment methodology applied in plants developed with Type 1 and Type 2 Site-Directed Nucleases and with oligonucleotide directed mutagenesis, has to consider (i) several distinct steps during the technical processes, (ii) the new combinations of genetic information and the resulting unintended and intended biological characteristics, as well as (iii) on-target and off-target effects caused by the activities of the biological mutagens. The set of data needed for risk assessment might in many cases substantially deviate from those described in EFSA (2012a). For example, if the newly generated gene combination results in profound changes in plant metabolism, the comparative risk assessment may be challenged to an extent that goes far beyond existing experience with transgenic plants or future SDN-3 applications. Also in regard to environmental risk assessment, there are new challenges that were not considered by EFSA (2012a) and may even go beyond current experience with transgenic plants orSDN-3 applications. These include changes in the composition of plants that may impact the food web, changes in the composition of plants that may impact plant communication and interaction with the environment, changes in the biological characteristics of the plants that concern their invasiveness and next generation effects of plants with the potential to persist and propagate in the environment (see Testbiotech, 2020).	the proposed changes have not been integrated in the text of the opinion. The GMO Panel considers that the conclusions are in line and consistent with the argumentations expressed in this opinion.	

		Since these issues were not considered in the EFSA (2012a), the methodology as described in EFSA (2012a) cannot be considered to be adequate and is insufficient to generate reliable conclusions on risk assessment. Since in regard to ODM, many data are missing, no final conclusion can be derived. In relation to ToR2, the GMO Panel concludes that the existing guidance and regulations for food and feed (EFSA, 2011; EU Commission, 2013) and environmental risk assessment (EFSA, 2010) are only partially sufficient. Due to the high potential to penetrate the genome and cause profound alterations in the biological characteristics of plants without introducing any additional DNA sequences, the current approach of comparative risk assessment will in many cases not be sufficient. In the absence of adequate comparators, new methods of risk assessment, such as whole genome sequencing, but also metabolomics, proteomics and transcriptomics might be needed to perform sufficiently robust risk assessment. Since in regard to ODM, many relevant data are missing, no final conclusion can be derived. Whatever the case, detailed examination of an organism's genetic and overall biological characteristics, starting with the process that was used to introduce changes in the genome of the organism, is needed to decide whether the organism is safe. The set of data needed for risk assessment will be dependent on each case and cannot generally be limited by criteria such as the insertion of additional genes."		
Umweltbundesamt (Environment Agency Austria) on behalf of the Austrian lead Competent Authority, the Federal Ministry of Social Affairs, Health, Care and Consumer Protection.	4. Conclusions	We recommend that the whole draft opinion and the conclusions are thoroughly revised and further developed to represent an applicable guidance for the risk assessment of SDN-1, SDN-2 and ODM applications reflecting the current scientific knowledge (see e.g. Eckerstorfer et al., 2019a; Modrzejewski et al., 2019; Kawall, 2019) as well as the regulatory experience with those technologies (e.g. Eckerstorfer et al., 2019b, USDA-APHIS, 2020). As indicated in our general comments the draft opinion needs to be developed into a stand-alone guidance for such applications, which is providing an appropriate case-specific discussion of the various different SDN-based techniques and outcomes and outline concrete requirements for a focused risk assessment. The discussion of case-specific assessment approaches should be based on an analysis of the characteristics of (available) examples to better illustrate the specific requirements and recommendations regarding the respective risk issues. The current draft conclusions are not addressing the subject appropriately. For the necessary revision our comments submitted to the draft opinion "Evaluation of existing guidelines for their adequacy for the molecular characterisation and environmental risk assessment of genetically modified plants obtained through synthetic biology" published by EFSA for public consultation on 31st March 2020 should be considered as well. Eckerstorfer, M. F., Dolezel, M., Heissenberger, A., Miklau, M., Reichenbecher, W., Stein-brecher, R. A., and Waßmann, F. (2019a). An EU Perspective on Biosafety Considera-tions for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs). Frontiers in bioengineering and biotechnology 7. doi: 10.3389/fbioe.2019.00031. Eckerstorfer, M. F., Engelhard, M., Heissenberger, A., Simon, S., and Teichmann, H. (2019b). Plants Developed by New Genetic Modification Techniques-Comparison of Ex-isting Regulatory Frameworks in the EU and Non-EU Countries. Frontiers in bioengineer-ing and biotechnology 7. doi:	The GMO Panel takes note of the comment. The opinion has been developed by strictly adhering to the terms of reference provided by the European Commission. The GMO Panel was not mandated neither to revise the current guidances nor to develop new ones for the risk assessment of plants generated via SDN-1, SDN-2, and ODM. Nevertheless, the GMO Panel takes note of the comment.	290

		10.3389/fbioe.2019.00026. Kawall, K. (2019). New Possibilities on the Horizon: Genome Editing Makes the Whole Genome Accessible for Changes. Frontiers in plant science 10. doi: 10.3389/fpls.2019.00525. Modrzejewski, D., Hartung, F., Sprink, T., Krause, D., Kohl, C., and Wilhelm, R. (2019). What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target ef-fects: a systematic map. Environ Evid 8 (1). doi: 10.1186/s13750-019-0171-5. USDA-APHIS (2020): Amendment of 7 CFR Parts 330, 340, and 372, Docket No. APHIS-2018-0034, RIN 0579-AE47		
International Seed Federation	4. Conclusions	ISF agrees with the conclusions of the GMO Panel. In addition, ISF would like to make the point that SDN1/2 technologies are part of the breeding cycle and integrated into classical breeding steps. Well-established plant breeding and selection practices, applied for any new plant variety development regardless of the breeding method, effectively identify and remove off-type plants while retaining plants with intended characteristics. These processes and plants have a long history of safe development. EFSA should stress the importance of the principle of proportionality as set out in Article 5 of the EU Treaty. According to this, EFSA would be expected to ensure that its measures and requests are appropriate and non-discriminatory to achieve the overall objective of safety, and do not go beyond what is necessary to achieve that goal. Therefore, any EFSA risk assessments conducted for plants developed using SDN-1, SDN-2 and ODM should be placed in context with similar plants developed through conventional breeding and not be subject to undergo a more stringent risk assessment, including the provision of extensive experimental data, merely because of the method used for their development.	The GMO Panel thanks and takes note of the comment.	291
Sciensano	4. Conclusions	We can agree with the conclusions of EFSA concerning the hazards (it is unlikely that SDN-1, SDN-2 and ODM will pose new hazards compared to SDN-3 and conventional breeding) and the partial applicability of the SDN-3 and existing EFSA guidance documents for these three techniques.	The GMO Panel thanks and takes note of the comment.	292
Agriculture and Food Systems Institute (AFSI)	4. Conclusions	To whom it may concern, These comments are being submitted on behalf of the Agriculture & Food Systems Institute (AFSI). AFSI is a non-profit scientific institute with a mission to achieve safe and sustainable agri-food systems by providing thought leadership and creating a collaborative environment that fosters scientific innovation across disciplines, sectors, and geographies. AFSI has a significant portfolio of analytical research relevant to environmental risk assessment and food safety assessment for products of biotechnology including plants, animals, arthropods and microorganisms. Additionally, AFSI is engaged in knowledge sharing internationally through its biosafety capacity building programs, and the provision of open data resources that are used by regulators, risk assessors and others in the scientific community. We would like to thank EFSA and the GMO panel for the opportunity to comment on this scientific opinion. Reviewing the opinion "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," we find the opinion to be fundamentally sound, demonstrating a logical process of analysis. The conclusions are consistent with best practices in environmental risk assessment and are grounded in relevant science.	The GMO Panel thanks and takes note of the comments.	293

		The opinion confirms SDN-1, SDN-2 and ODM techniques are employed to produce plants that may not have the characteristics of GMOs anticipated by Directive 2001/18/EC i.e., the introduction of DNA sequences from other organisms that lead to the expression of proteins or gene products not previously present in the plant species. The opinion of the panel that data requirements should be flexibly applied in acknowledgement of this fact is consistent with the underlying science as well as case by case risk assessment methodology. While the language and terminology in the document is generally clear and appropriate, there is some inconsistency in the way the term "hazard" is used. In particular, with respect to the potential for hazards to arise from off-target mutations or changes in the genome of a plant developed using SDN1, SDN2 or ODM techniques, the opinion often describes these molecular effects simply as "hazards". It is important to acknowledge that changes to genomes – even those which affect protein expression or function – are not inherently hazardous and, in fact, most genomic changes are not expected to produce hazards. Genetic changes are not, in and of themselves, "hazards" as they are described throughout the document. This is amply demonstrated by extensive experience with conventional mutagenesis methods which produce considerably more genomic changes which are rarely associated with hazards. The language in the opinion should be revised to consistently identify that genomic changes are potential hazards that may need to be assessed.		
Federal Agency for Nature Conservation	4. Conclusions	The draft focuses on the question whether SDN-1, SDN-2 and ODM interventions – like SDN-3 – insert foreign genes or not. However, it disregards the present and upcoming potentials and possibilities especially of SDN-1 and SDN-2 for deep genomic interventions. Also, the draft disregards that conventional breeding and genome editing take two distinct approaches to achieve a new trait. Because of this narrow approach, we do not share the overall conclusion that no new and specific hazards specifically linked to the genomic modification produced via SDN-1, SDN-2 and ODM as compared to both SDN-3 and conventional breeding were identified.	The GMO Panel takes note of the comment. Regarding the aspects related to "deep genomic intervention" and "conventional breeding", the GMO Panel refers the contributor to the related comments and the responses provided throughout the document.	294
Envirnonmental association Za Zemiata	4. Conclusions	As a general comment, this draft Opinion appears to seek to minimise recognition of any errors created by genome editing. It does not do EFSA credit. Errors such as exon skipping and off target effects require rigorous assessment if genome-edited crops are to be considered for use in agriculture. The Opinion appears to focus on the most recent techniques such as "DNA-free" genome editing, when the reality is that those created by insertion of transgenes coding for genome editing components are more likely to be the subject of applications for deliberate release and marketing in the first instance. [line 412-425 delete and replace:] "In relation to ToR1, the GMO Panel concludes that the assessment methodology presented in section 4 of the EFSA opinion on SDN-3 is partially applicable to SDN-1, SDN-2, and ODM. However, EFSA (2012a) did not provide a scientific basis for conventional plant breeding (using non-targeted chemical or physical mutagens) being compared to methods of genetic engineering using targeted biological mutagens. Therefore, EFSA (2012a) methodology has fundamental deficiencies. Beyond that, EFSA (2012a) did not consider the potential of SDN-1 and SDN-2 applications to penetrate the genome and cause profound alterations in the biological characteristics of plants	The GMO Panel considers that for the comments related to lines 412-425, an explanation of the rationale for the proposed change is insufficient. Therefore, the proposed changes have not been integrated in the text of the opinion. The GMO Panel considers that the conclusions are in line and consistent with the argumentations expressed in this opinion.	295

		without introducing any additional DNA sequences. As shown, risk assessment methodology applied in plants developed with Type 1 and Type 2 Site-Directed Nucleases and with oligonucleotide directed mutagenesis, has to consider (i) several distinct steps during the technical processes, (ii) the new combinations of genetic information and the resulting unintended and intended biological characteristics, as well as (iii) on-target and off-target effects caused by the activities of the biological mutagens. The set of data needed for risk assessment might in many cases substantially deviate from those described in EFSA (2012a). For example, if the newly generated gene combination results in profound changes in plant metabolism, the comparative risk assessment may be challenged to an extent that goes far beyond existing experience with transgenic plants or future SDN-3 applications. Also in regard to environmental risk assessment, there are new challenges that were not considered by EFSA (2012a) and may even go beyond current experience with transgenic plants orSDN-3 applications. These include changes in the composition of plants that may impact the food web, changes in the composition of plants that may impact plant communication and interaction with the environment, changes in the biological characteristics of the plants that concern their invasiveness and next generation effects of plants with the potential to persist and propagate in the environment (see Testbiotech, 2020). Since these issues were not considered in the EFSA (2012a), the methodology as described in EFSA (2012a) cannot be considered to be adequate and is insufficient to generate reliable conclusions on risk assessment. Since in regard to ODM, many data are missing, no final conclusion can be derived. In relation to ToR2, the GMO Panel concludes that the existing guidance and regulations for food and feed (EFSA, 2011; EU Commission, 2013) and environmental risk assessment (EFSA, 2010) are only partially sufficient. Due to the high potential to penetrate the genome and cause profound alterations in the biological characteristics of plants without introducing any additional DNA sequences, the current approach of comparative risk assessment will in many cases not be sufficient. In the absence of adequate comparators, new methods of risk assessment, such as whole genome sequencing, but also metabolomics, proteomics and transcriptomics might be needed to perform sufficiently robust risk assessment. Since in regard to ODM, many relevant data are missing, no final conclusion can be derived. Whatever the case, detailed examination of an organism's genetic and overall biological characteristics, starting with the process that was used to introduce changes in the genome of the organism, is needed to decide whether the organism is safe. The set of data needed for risk assessment will be dependent on each case and cannot generally be limited by criteria such as the insertion of additional genes."		
Corporate Europe Observatory	4. Conclusions	The draft Opinion appears to ignore many studies on off-target and on-target effects which are very relevant to food/feed and environmental safety. If EFSA chooses to not consider the studies mentioned in this submission, we request an explanation for this.	To develop the opinion, the GMO panel not only evaluated review and opinion papers but also	296

		Risk assessment methodology for SDN1, SDN2 and ODM has to consider, inter alia, the different steps of the technical processes, the new combinations of genetic information, the resulting (unintended and intended) characteristics, on-target and off-target effects.	research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. Therefore, the GMO Panel considers the conclusions in line and consistent with the argumentations expressed in this opinion.	
BUND e.V. / Friends of the Earth Germany	4. Conclusions	Line 421 to 422 DELETE from "but.." incl. "Indeed." Line 425 ADD final sentence. "This does not affect the need for a broad risk assessment, though." General comment: This whole section needs revision. We refer to our previous comments citing literature that shows specific risks related to the use of CRISPR/Cas, such as on-target and off-target effects (Kapahnke et al. 2016, Lalonde et al. 2017, Mou et al. 2017, Smits et al. 2019, Hahn und Nekrasov 2019, Murugan et al. 2020), which have to be addressed and assessed in future. There is a wide range of open questions connected with the use of SDN-1 and SDN-2 applications as well, they must be addressed sufficiently in any recommendation on risk assessment.	The GMO Panel considers that for the comments related to lines 421-422, and 425, 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. Regarding the aspects on "on-target" and "off-target" effects, the GMO Panel refers the contributor to the related comments and the responses provided (section 3.2 of the document).	297
CropLife Canada	4. Conclusions	CropLife Canada agrees with the conclusion of the EFSA GMO Panel that plants developed using SDN-1, SDN-2 and ODM approaches are unlikely to represent any additional hazards compared with SDN-3 and conventional breeding techniques, including mutagenesis. If available, these technologies will become part of the breeding cycle and integrated into well-established classical breeding and selection practices, applied for any new plant variety development regardless of the breeding method, to effectively identify and remove off-type plants while retaining plants with intended characteristics. As such, these technologies should leverage the long safe history of similar products resulting from conventional breeding which should provide the basis for proportional type of risk assessment. While using the case-by-case approach and the application of problem formulation is useful in guiding the assessment to establish what data requirements are relevant, it will be important to establish some clear guideposts so that requirements are consistent from product to product don't default closer to the full type risk assessment.	The GMO Panel thanks CropLife Canada for the comment.	298
ENSSER	5. Glossary	SDN module	The GMO Panel thanks for the comment. The term has been added to the glossary.	299

Association Française de Biotechnologies Végétales	5. Glossary	AFBV edit: Line 441: replace "alteration" by "change".	The GMO Panel thanks for the comments. The text has been revised accordingly.	300
Julius Kühn-Institut	5. Glossary	L444: NHEJ does not lead to genomic mutations in most cases. Therefore, write: "In some cases NHEJ results in genomic mutations, …". In case of SDN-1 the likelihood to achieve a mutation is increased because the SDN will identify the site as target as long as the sequence has not been modified and hence a DSB and NHEJ may repeatedly be induced. L446-447: Off-target mutations are not a specific issue with genome editing and may result from different methods employed for breeding. Omit "as a result of the application of genome editing techniques". L448: Oligonucleotides may not only consist of DNA. Hence replace "DNA" by "NA"	Regarding comment to line 444, the text has been amended accordingly. Regarding comment to line 446-447, the GMO Panel considers the definition in the glossary appropriate in the context of this opinion. Regarding comment to line 448, the text has been amended accordingly	301
French agency for Food, Environmental and Occupational Health & Safety (Anses)	5. Glossary	Page 13, lines 428-458: Proposal to have the Glossary section at the beginning of the document, for instance after the Keywords section, to make the document easier to understand. The addition of a section containing the abbreviations before the glossary would be helpful.	The GMO Panel thanks for the suggestion. The glossary section is included at the end of the document to follow the EFSA publications' guideline.	302
Corteva Agriscience	5. Glossary	Line 430, CRISPR definition: typo; change "clusters of" to "clustered". Line 443, NHEJ definition: Change "NHEJ results in genomic mutation" to "NHEJ can result in genomic mutation". NHEJ can be a seamless repair as well, it is just error prone (Rodgers K. and Mcvey M. (2016) Error-prone repair of DNA double-strand breaks. J Cell Physiol. 231(1): 15–24). Line 456, transgene definition. Confusing use of the term "exogenous". Suggest defining as "a gene from a different, sexually-incompatible species". Line 457, transgenesis definition. As above, suggest changing to "gene(s) from a different, sexually-incompatible species".	The GMO Panel thanks for the comments. The text has been revised accordingly.	303
European Plant Science Organisation, EPSO	5. Glossary	Line 444: Non-homologous end joining: NHEJ may result in perfect repair or in genomic mutations, usually these are insertions or deletions of a small number of nucleotides. Line 446: Off-target mutation: It should be noted that off-target mutations are not specific to genome editing techniques and may result from different breeding methods. We suggest deleting "as a result of the application of genome editing techniques"	The GMO Panel thanks for the comments. The text has been revised accordingly. Regarding the definition of off-targets, the GMO Panel considers the definition in the glossary appropriate in the context of this opinion.	304

		Line 448: Oligonucleotides could also consist of RNA or LNA. We suggest replacing "DNA" by "nucleic acid" or to write "Oligonucleotide: a stretch of DNA, RNA or Locked Nucleic Acid (LNA) consisting of a relatively low number of nucleotides".		
Haut Conseil des biotechnologies (High Council for Biotechnology)	5. Glossary	l. 433. "enzymatic" instead of "enzymatical". l. 437. "organism" instead of "organisms" l. 440 and l. 444. "a homologous" instead of "an homologous" l. 458. "propagation" unclear.	The GMO Panel thanks for the comments. The text has been revised accordingly.	305
ENSSER	6. Reference	• Agapito-Tenfen SZ, Okoli AS, Bernstein MJ, Wikmark OG, Myhr AI (2018) Revisiting risk governance of GM plants: The need to consider new and emerging gene-editing techniques. Front Plant Sci 9:1874. doi:10.3389/fpls.2018.01874 • Akcakaya et al. (2018). "In vivo CRISPR editing with no detectable genome-wide off-target mutations." Nature 561 (7723):416-+. doi: 10.1038/s41586-018-0500-9 • Bertheau, Yves. (2019). New Breeding Techniques: Detection and Identification of the Techniques and Derived Products. 10.1016/B978-0-08-100596-5.21834-9. • Chen K, Wang Y, Zhang R, Zhang H, Gao C (2019) CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture. Annu Rev Plant Biol 70:667-697. doi:10.1146/annurev-arplant-050718-100049 • Eckerstorfer, M. F., Dolezel, M., Heissenberger, A., Miklau, M., Reichenbecher, W., Steinbrecher, R. A., Wassmann, F. (2019). An EU perspective on biosafety considerations for plants developed by genome editing and other new genetic modification techniques (nGMs). Front Bioeng Biotechnol, 7, 31. doi:10.3389/fbioe.2019.00031 • Hajiahmadi, Z., Movahedi, A., Wei, H., Li, D., Orooji, Y., Ruan, H., & Zhuge, Q. (2019). Strategies to Increase On-Target and Reduce Off-Target Effects of the CRISPR/Cas9 System in Plants. International journal of molecular sciences, 20(15), 3719. https://doi.org/10.3390/ijms20153719 • Jeon J et al. (2019). Retroelement Insertion in a CRISPR/Cas9 Editing Site in the Early Embryo Intensifies Genetic Mosaicism. Front. Cell Dev. Biol. 7:273. doi: 10.3389/fcell.2019.00273 • Kannan B, Jung JH, Moxley GW, Lee SM, Altpeter F (2018) TALEN-mediated targeted mutagenesis of more than 100 COMT copies/alleles in highly polyploid sugarcane improves saccharification efficiency without compromising biomass yield. Plant Biotechnol J 16 (4):856-866. doi:10.1111/pbi.12833 • Kawall K (2019) New Possibilities on the Horizon: Genome Editing Makes the Whole Genome Accessible for Changes. Front Plant Sci 10:525. doi:10.3389/fpls.2019.00525 • Kawall K, Cotter J, Then C (2020) Broadening the GMO risk assessment in the EU for genome editing technologies in agriculture. Env Sci Eur (in press) • Kosicki, M., K. Tomberg, and A. Bradley. (2018.) "Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and com¬plex rearrangements." Nature Biotechnology 36 (8):765-+. doi: 10.1038/nbt.4192. • Modrzejewski, D., Hartung, F., Sprink, T. et al. What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a systematic map. Environ Evid 8, 27 (2019). https://doi.org/10.1186/s13750-019-0171-5 • Vanessa E. Prescott, Peter M. Campbell, Andrew Moore, Joerg Mattes, Marc E. Rothenberg, Paul S. Foster, T. J. V. Higgins, and Simon P. Hogan Transgenic Expression of Bean α-Amylase Inhibitor in	The GMO Panel takes note of the list of references provided in the comment.	306

		Peas Results in Altered Structure and Immunogenicity Journal of Agricultural and Food Chemistry 2005 53 (23), 9023-9030. DOI: 10.1021/jf050594v • Sanchez-Leon S, Gil-Humanes J, Ozuna CV, Gimenez MJ, Sousa C, Voytas DF, Barro F (2018) Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. Plant Biotechnol J 16 (4):902-910. doi:10.1111/pbi.12837 • Tuladhar R, Yeu Y, Tyler Piazza J, Tan Z, Rene Clemenceau J, Wu X, Barrett Q, Herbert J, Mathews DH, Kim J, Hyun Hwang T, Lum L (2019) CRISPR-Cas9-based mutagenesis frequently provokes on-target mRNA misregulation. Nat Commun 10 (1):4056. doi:10.1038/s41467-019-12028-5 • Zhang Y, Massel K, Godwin ID, Gao C (2018) Applications and potential of genome editing in crop improvement. Genome Biol 19 (1):210. doi:10.1186/s13059-018-1586-y • Zsogon A, Cermak T, Naves ER, Notini MM, Edel KH, Weinl S, Freschi L, Voytas DF, Kudla J, Peres LEP (2018) De novo domestication of wild tomato using genome editing. Nat Biotechnol. doi:10.1038/nbt.4272		
Association Française de Biotechnologies Végétales	6. Reference	AFBV comment: Line 566: AFBV suggests to add the following reference: POMPILI, V., COSTA, L., PIAZZA, S., PINDO, M., & MALNOY, M. 2020. Reduced fire blight susceptibility in apple cultivars using a high-efficiency CRISPR/Cas9-FLP/FRT-based gene editing system. Plant Biotechnology Journal, 18, 845–858. This reference is cited in the addition proposed on Table 1, paragraph 3.1.3.	Please note that Table 1 has been removed from the document.	307
Euroseeds	6. Reference	Please include the additional references as referenced in our contribution and as provided in the upload file.	The GMO Panel thanks for the comment. Please refer to the panel's responses to the related comments in the other sections of the opinion.	308
COST Action CA18111 - Plant genome editing – a technology with transformative potential (PlantEd)	6. Reference	EFSA (2012). Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function. EFSA J. 10. doi:10.2903/j.efsa.2012.2943. EFSA (2020). Applicability of the EFSA opinion on site-directed nucleases type for the safety assessment of plants developed using site-directed nucleases type and and oligonucleotide-directed mutagenesis. 16 pp. Available at: https://www.efsa.europa.eu/en/consultations/call/public-consultation-applicability-efsa-opinion-site-directed [Accessed May 5, 2020]. Deng, H., Huang, W., & Zhang, Z. (2019). Nanotechnology based CRISPR/Cas9 system delivery for genome editing: Progress and prospect. Nano Research, 1-14. DONG, C. , BEETHAM, P. , VINCENT, K. AND SHARP, P. (2006) Oligonucleotide- directed gene repair in wheat using a transient plasmid gene repair assay system. Plant Cell Rep. 25, 457–465. GOCAL, G.F.W. , SCHÖPKE, C. AND BEETHAM, P.R. (2015) Oligo- mediated targeted gene editing In Advances in New Technology for Targeted Modification of Plant Genomes Chapter 5 (Zhang F., Puchta H. and Thomson J.G.,eds), pp. 73–90. Berlin, Heidelberg: Springer Verlag. COLE- STRAUSS, A. , YOON, K. , XIANG, Y. , BYRNE, B.C. , RICE, M.C. , GRYN, J. , HOLLOMAN, W.K. et al (1996) Correction of the mutations responsible for sickle cell anemia by an RNA- DNA	The GMO Panel takes note of the list of references provided in the comment.	309

		oligonucleotide. Science, 273, 1386–1389 ALEXEEV, V. AND YOON, K. (1998) Stable and inheritable changes in genotype and phenotype of albino melanocytes induced by an RNA- DNA oligonucleotide. Nat. Biotechnol. 16, 1343–1346. BEETHAM, P.R. , KIPP, P.B. , SAWYCKY, X.L. , ARNTZEN, C.J. AND MAY, G.D. (1999) A tool for functional plant genomics: chimeric RNA/DNA oligonucleotides cause in vivo gene- specific mutations. Proc. Natl Acad. Sci. USA, 96, 8774–8778 Leyser, O. (2018). GM crop ruling shows why the EU' s laws are wholly inadequate. Conversat. Available at: https://theconversation.com/gm-crop-ruling-shows-why-the-eus-laws-are-wholly-inadequate-100675 [Accessed May 17, 2019]. Purnhagen, K. P., Kok, E., Kleter, G., Schebesta, H., Visser, R. G. F., and Wesseler, J. (2018). EU court casts new plant breeding techniques into regulatory limbo. Nat. Biotechnol. 36, 799–800. doi:10.1038/nbt.4251. SAUER, N. J., MOZORUK, J., MILLER, R. B., WARBURG, Z. J., WALKER, K. A., BEETHAM, P. R., SCHOPKE, 566 C. R. & GOCAL, G. F. W. 2016. Oligonucleotide-directed mutagenesis for precision gene 567 editing. Plant Biotechnology Journal, 14, 496-502 Sprink, T., Eriksson, D., Schiemann, J., and Hartung, F. (2016). Regulatory hurdles for genome editing: process- vs. product-based approaches in different regulatory contexts. Plant Cell Rep. 35, 1493–1506. doi:10.1007/s00299-016-1990-2. Vives-Vallés, J. A., and Collonnier, C. (2020). The Judgment of the CJEU of 25 July 2018 on Mutagenesis: Interpretation and Interim Legislative Proposal. Front. Plant Sci. 10, 1813. doi:10.3389/fpls.2019.01813. Zhang, Y., Malzahn, A. A., Sretenovic, S., & Qi, Y. (2019). The emerging and uncultivated potential of CRISPR technology in plant science. Nature plants, 5(8), 778-794.		
French agency for Food, Environmental and Occupational Health & Safety (Anses)	6. Reference	Page 14, line 460, "6 Reference": please write it in the plural form (6 References").	The text has been amended accordingly.	310
Corteva	6. Reference	Suggest to add reference to a recently published paper: Off-target changes in plant genome editing Nathaniel Graham, Gunvant Patil, David M Bubeck, Raymond C Dobert, Kevin C Glenn, Annie T Gutsche, Sandeep Kumar, John A Lindbo, Luis Maas, Gregory D May, Miguel E Vega-Sanchez, Robert M Stupar, Peter L Morrell	The GMO Panel takes note of the references suggested in the comment.	311

		Plant Physiology May 2020, pp.01194.2019; DOI: 10.1104/pp.19.01194 http://www.plantphysiol.org/content/early/2020/05/26/pp.19.01194		
Testbiotech	6. Reference	References are attached in additional file (uploaded)	The GMO Panel takes note of the list of references provided.	312
Federal Agency for Nature Conservation	6. Reference	Agapito-Tenfen, Sarah Z.; Okoli, Arinze S.; Bernstein, Michael J.; Wikmark, Odd-Gunnar; Myhr, Anne I. (2018): Revisiting Risk Governance of GM Plants: The Need to Consider New and Emerging Gene-Editing Techniques. In: Frontiers in plant science 9, p. 1874. DOI: 10.3389/fpls.2018.01874. Beying, N., Schmidt, C., Pacher, M. et al. CRISPR–Cas9-mediated induction of heritable chromosomal translocations in Arabidopsis. Nat. Plants (2020). https://doi.org/10.1038/s41477-020-0663-x Eckerstorfer, Michael F.; Heissenberger, Andreas; Reichenbecher, Wolfram; Steinbrecher, Ricarda A.; Waßmann, Friedrich (2019): An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs). In: Frontiers in bioengineering and biotechnology 7, p. 319. DOI: 10.3389/fbioe.2019.00031. Hahn, Florian; Nekrasov, Vladimir (2019): CRISPR/Cas precision: do we need to worry about off-targeting in plants? In: Plant Cell Rep 38 (4), p. 437–441. DOI: 10.1007/s00299-018-2355-9. Kannan, Baskaran; Jung, Je Hyeong; Moxley, Geoffrey W.; Lee, Sun-Mi; Altpeter, Fredy (2018): TALEN-mediated targeted mutagen-esis of more than 100 COMT copies/alleles in highly polyploid sugarcane improves saccharification efficiency without com-promising biomass yield. In: Plant Biotechnol J 16 (4), p. 856–866. DOI: 10.1111/pbi.12833. Kapahnke, Marcel; Banning, Antje; Tikkanen, Ritva (2016): Random Splicing of Several Exons Caused by a Single Base Change in the Target Exon of CRISPR/Cas9 Mediated Gene Knockout. In: Cells 5 (4). DOI: 10.3390/cells5040045. Kawall, Katharina (2019): New Possibilities on the Horizon: Genome Editing Makes the Whole Genome Accessible for Changes. In: Front. Plant Sci. 10, p. 280. DOI: 10.3389/fpls.2019.00525. Kosicki, M., Tomberg, K. & Bradley, A. Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. Nat. Biotechnol. https://doi.org/10.1038/nbt.4192 (2018). Modrzejewski, Dominik; Hartung, Frank; Sprink, Thorben; Krause, Dörthe; Kohl, Christian; Wilhelm, Ralf (2019): What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a systematic map. In: Environmental Evidence 8 (1), p. 27. DOI: 10.1186/s13750-019-0171-5. Mou, Haiwei; Smith, Jordan L.; Peng, Lingtao; Yin, Hao; Moore, Jill; Zhang, Xiao-Ou et al. (2017): CRISPR/Cas9-mediated genome editing induces exon skipping by alternative splicing or exon deletion. In: Genome biology 18 (1), p. 108. DOI: 10.1186/s13059-017-1237-8.	The GMO Panel takes note of the list of references provided in the comment.	313

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Envirnonmental association Za Zemiata	6. Reference	Agapito-Tenfen, S. Z., Okoli, A. S., Bernstein, M. J., Wikmark, O. G., & Myhr, A. I. (2018) Revisiting risk governance of GM plants: The need to consider new and emerging gene-editing techniques. Front Plant Sci, 9, 1874. doi:10.3389/fpls.2018.01874 Bauer-Panskus, A., Miyazaki, J., Kawall, K., Then, C. (2020) Risk assessment of genetically engineered plants that can persist and propagate in the environment. Environ Sci Eur 32, 32. doi: 10.1186/s12302-020-00301-0 Braatz, J., Harloff, H. J., Mascher, M., Stein, N., Himmelbach, A., and Jung, C. (2017) CRISPR-Cas9 targeted mutagenesis leads to simultaneous modification of different homoeologous gene copies in polyploid oilseed rape (Brassica napus). Plant Physiol, 174, 935–942. doi:10.1104/pp.17.00426 Bonner, M., Strouse, B., Applegate, M., Livingston, P., & Kmiec, E. B. (2012). DNA damage response pathway and replication fork stress during oligonucleotide directed gene editing. Mol Ther Nucleic Acids, 1, e18. doi: 10.1038/mtna.2012.9 Brinkman, E.K., Chen, T., de Haas, M., Holland, H.A., Akhtar, W., van Steensel, B. (2018) Kinetics and fidelity of the repair of Cas9-induced double-strand DNA breaks. Molecular Cell, 70(5): 801-813 e806. https://www.sciencedirect.com/science/article/pii/S1097276518303125 COGEM (2019) Gene Edited Crops; Global Perspectives and Regulation, Report of the COGEM International Symposium, https://cogem.net/app/uploads/2020/01/200115-01-COGEM-symposium-gene-edited-crops-global-perspectives-regulation-1.pdf Conseil d'État (2020) 7 février 2020, Organismes obtenus par mutagenèse, https://www.conseil-etat.fr/ressources/decisions-contentieuses/dernieres-decisions-importantes/conseil-d-etat-7-fevrier-2020-organismes-obtenus-par-mutagenese Cotter, J., Kawall K., Then C., (2020) New genetic engineering technologies, RAGES Report, www.testbiotech.org/en/content/rages-subreport-new-genetic-engineering-technologies Ding, X., Seebeck, T., Feng, Y., Jiang, Y., Davis, G.D., Chen, F. (2019) Improving CRISPR-Cas9 Genome Editing Efficiency by Fusion with Chromatin-Modulating Peptides. CRISPR J 2, 51-63. Dong, C., Beetham, P., Vincent, K., & Sharp, P. (2006). Oligonucleotide-directed gene repair in wheat using a transient plasmid gene repair assay system. Plant Cell Rep, 25(5), 457-465. doi: 10.1007/s00299-005-0098-x Duensing, N., Sprink, T., Parrott, W. A., Fedorova, M., Lema, M. A., Wolt, J. D., Bartsch, D. (2018) Novel features and considerations for ERA and regulation of crops produced by genome editing, Front Bioeng Biotechnol 6:79., doi: 10.3389/fbioe.2018.00079 Eckerstorfer, M. F., Dolezel, M., Heissenberger, A., Miklau, M., Reichenbecher, W., Steinbrecher, R. A., Wassmann, F. (2019). An EU perspective on biosafety considerations for plants developed by	The GMO Panel takes note of the list of references provided in the comment.	314

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EuropaBio	Other comments	General Comments • EuropaBio welcomes the opportunity to comment on this document and agrees with the main conclusion of the EFSA GMO Panel that plants obtained using SDN-1, SDN-2, and ODM are unlikely to pose any additional hazards compared to those obtained using SDN-3 and conventional breeding techniques including mutagenesis. • EuropaBio agrees that it is not necessary to introduce new requirements to assess plants obtained using SDN-1, SDN-2, and ODM. We consider that there are aspects of the risk assessment methodology presented in the EFSA opinion on SDN-3 that are not applicable to plants produced using SDN-1, SDN-2 and ODM, if the final product does not contain any exogenous DNA. EuropaBio is of the opinion that the case-by-case approach and problem formulation methodology described in existing guidance would be sufficient to determine the relevance of data requirements for each product. For data requirements not relevant for a particular product, EFSA should implement the use of the derogation clause in IR 503/2013. • EuropaBio suggests that, in view of the fact that plants developed using SDN-1, SDN-2 and ODM approaches often result in similar products to those obtained by conventional breeding, EFSA should stress the importance of the principle of proportionality as set out in Article 5 of the EU Treaty (From farm to fork: the regulatory status of non-GMO plant innovations under current EU law, Bioscience Law Rev. VOL 16 ISSUE 6 (2018)). According to this, EFSA would be expected to ensure that its	The GMO Panel thanks EuropaBio for the comments. The GMO Panel takes note of the comments.	316

		measures and requests are appropriate and non-discriminatory to achieve the overall objective of safety, and do not go beyond what is necessary to achieve that goal. As per line 154 "hazards regarding these alterations may arise both in conventional breeding and in transgenesis", any risk assessments conducted for plants developed using SDN-1, SDN-2 and ODM should be placed in context with similar plants developed through conventional breeding and not subject to more stringent risk assessment than conventionally bred plants, including the provision of extensive experimental data, merely because of the method used for their development. • EuropaBio agrees with the conclusion of the EFSA GMO Panel that the analysis of potential off-target changes in plants developed using SDN and ODM approaches is of "very limited value for the risk analysis". • EuropaBio considers that, as was suggested in the recent EFSA draft opinion for gene drives (http://www.efsa.europa.eu/en/consultations/call/public-consultation-gmo-panel-scientific-opinion-evaluation), pre-submission consultations and the facilitation of dialogue between EFSA and applicants would be of key importance to determine the best approach to follow regarding data generation for SDN and ODM products to ensure fit-for-purpose risk assessments. • EuropaBio considers that the principle of proportionality should be applied and the risk assessment should focus on the final trait(s) and the hazards that these may pose. Conducting the risk assessment for each single may not be practical, nor useful for the overall risk analysis of the intended final product. According to the CIR 503/2013, for stacked transgenic plant products there is a requirement to provide a risk assessment of each single transformation event before the stacked product can be assessed. EuropaBio considers that if the commercial product has been produced by conventional breeding crosses of single plants developed by SDN-1, SDN-2 and ODM approaches, but none of those edited plants are going to be commercialized alone, it does not make sense to perform a risk assessment for each of the edits before the combination can be assessed. Furthermore, when previously evaluated beneficial mutations are combined through conventional crossing there should be no need for additional assessment of combined products, except in rare cases where credible risks are defined by the problem formulation.		
Norwegian Scientific Committee for Food and Environment (VKM)	Other comments	The value of this opinion for risk assessors would have been improved if the opinion discussed more thoroughly point-by-point which data requirements outlined in the Guidance for risk assessment of food and feed from genetically modified plants (EFSA, 2011) and the Guidance on the environmental risk assessment of genetically modified plants (EFSA, 2010) are relevant or not relevant for plants developed using the SDN-1, SDN-2 and ODM techniques.	The GMO Panel thanks for the comment but would like to clarify that the panel was not mandated to provide a point-by-point revision of the data requirements outlined in the two EFSA guidances.	317
Comisión Nacional de Bioseguridad, Ministerio para la Transición Ecológica y el Reto Demográfico (MITECO)	Other comments	The National Commission on Biosafety of Spain welcome EFSA report and we agrees with conclusion. However, we would like to make the following comments/suggestions: - Greater emphasis should be placed on the conclusions on the importance on the case by case approach and the principle of proportionality for the risk assessment and to evaluate the applicability of the EFSA guidance. This is particularly important when the final product does not contain an exogenous DNA (there are aspects of the common methodology which are not applicable to SDN-1,	The GMO Panel thanks for the comment but would like to clarify that the panel was not mandated to provide a complete list of all the requirements not applicable to risk assess genome edited plants. Given the variety of products	318

		SDN-2 and ODM). - To enhance clarity and usefulness of this complete report, we suggest to include in conclusions a list of those items/areas where updating would be recommended for the RA, including, for example, the specific requirements referring to the introduced transgenes (exogenous DNA) which are not applicable for certain products obtained with SDN-1, SDN-2 and ODM. It would be also interesting and helpful to link these items with Regulation 503/2013. This approach has been used in SynBio report.	achievable with genome editing techniques, the GMO Panel considers that the case-by-case approach, as described in the opinion on SDN-3, is also applicable to genome edited plants.	
Associazione Luca Coscioni per la libertà di ricerca scientifica	Other comments	ALC and SD agree with the EFSA risk assessment evaluation that confirms that SDN-1, SDN-2 and ODM site directed mutagenesis do not show additional risks from mutations obtained by conventional breeding techniques or mutagenesis. Nevertheless, some aspects could be improved. In particular the definition of "mutagenesis techniques which have conventionally been used in a number of applications and have a long safety record" present in the sentence of the ECJ C-528/16. SDN-1, SDN-2 2 and ODM do not show additional risks and should be equally evaluated as safe technology. Also, in the case of GMOs, these have really a long safety record (GMO crops have been used for almost 37 years now) but are heavily regulated.	The GMO Panel thanks ALC and SD for the comment. The sentence reported in the comment has been provided by the European Commission in the frame of the mandate and it has been reported as it is in this opinion. The GMO Panel was not mandated to express an opinion on how genome edited plants should be risk assessed but rather to assess the applicability of the section 4 and conclusions of the opinion on SDN-3 to plant obtained via SDN-1, SDN-2, and ODM.	319
Euroseeds	Other comments	Considering the importance of the topic as well as the above-mentioned conclusions, Euroseeds asks the EFSA GMO Panel to give the principle of proportionality a more prominent place in the evaluation. The principle of proportionality is set out in Article 5 of the EU Treaty (TEU)[1], and has been included in the General Food Law which states "In accordance with the principle of proportionality as set out in Article 5 of the Treaty, this Regulation does not go beyond what is necessary in order to achieve the objectives pursued" (Regulation (EC) No 178/2002) and thus it is a principle that needs to be kept in mind in the evaluation of every mandate. In accordance with the principle of proportionality, EFSA would be expected to ensure that its measures and requests are appropriate and non-discriminatory to achieve the overall objective of safety, and do not go beyond what is necessary to achieve that goal. Therefore, to create a level playing field, we ask that EFSA makes clearer, using a problem formulation, which requirements developed for transgenic plants or food and feed products resulting from those plants are essential to be applied to protect the European consumer, animals and the environment to the same level as for conventional bred crops. It cannot be considered proportionate that in case of two almost identical plants with similar risk profiles one product would need to undergo a stringent risk assessment merely because of the method used for its development. Regarding stacking/pyramiding of beneficial gene-edited alleles In addition to the comments made to the previous sections, we would like to raise the issue of the requirement of single first assessment that has been introduced for stacked transgenic events. As	The GMO Panel thanks Euroseed for the comments and takes note of them. The GMO Panel was not mandated to express an opinion on how genome edited plants should be risk assessed but rather to assess the applicability of the section 4 and conclusions of the opinion on SDN-3 to plant obtained via SDN-1, SDN-2, and ODM. The "principle of proportionality" and the aspect related to "stacking/pyramiding" in genome edited plants were not addressed in the opinion on SDN-3 and therefore were not specifically addressed in this opinion. However, given the variety of products achievable with genome editing techniques, the GMO Panel considers that the concept of case-	320

		companies ceased to submit applications for cultivation of (stacked) transgenic events, it might have become less clear to EU regulators that in many cases combining of different mode of actions for control of pests and diseases should be encouraged to delay resistance development. This is common practice in conventional plant breeding where pyramiding (stacking) disease resistance genes by laborious and time-consuming cross breeding approaches are used to achieve durable resistance and where phenotypic assessment of the effect of individual alleles is often not possible due to the lack of differentiating pathogen strains (17) . Resistance has been introgressed into wheat e.g. from at least 52 species from 13 genera, demonstrating the remarkable plasticity of the wheat genome and the importance of such natural variation in wheat breeding (18) . For crops produced using targeted mutagenesis approaches it might also be necessary to combine multiple adjusted disease resistance alleles in one or more steps and this should be encouraged by the regulatory framework to benefit from a delayed resistance formation of pests and pathogens. Therefore, this "single first assessment" should not be applicable as the risk assessment should focus on the stacked product that is intended to be commercialised. In addition, for polyploid crops it might be necessary to introduce similar or identical changes in the different alleles of the different genomes as well as in different alleles of one gene family. These genes are genetically unlinked but risk assessment of changes to one allele is useless if the expression of the trait depends on changes to all alleles in different genomes as it is the nature of recessive alleles that need to be present in homozygous form to achieve the expected phenotypic effect/trait also in conventional cross breeding . Furthermore, when previously evaluated beneficial mutations are combined through conventional crossing which is the overall aim of all conventional breeding efforts (combining beneficial alleles by cross breeding), there should be no need for additional assessment of stacked products, except in rare cases where credible risks are defined by the problem formulation. (17) see e.g. Strategies for pyramiding resistance genes against the Barley Yellow Mosaic Virus complex (BaMMV, BaYMV, BaYMV-2) 2005, Molecular Breeding 16(1):45-55, DOI: 10.1007/s11032-005-3445-2 (18) Strategies for transferring resistance into wheat: from wide crosses to GM cassettes, 2014; doi: 10.3389/fpls.2014.00692	by-case approach, as described in the opinion on SDN-3, is also applicable to genome edited plants.	
SETA (Science and Technology in Agriculture)	Other comments	SETA agrees with the EFSA risk assessment evaluation that confirms that SDN-1, SDN-2 and ODM site directed mutagenesis do not show additional risks compared to mutations obtained by conventional breeding techniques or mutagenesis in case foreign DNA is not present in the final product and this results in mutations in plant resident genomic sequences without the insertion of a long stretch of DNA. Despite this, many aspects might still be improved. In the judgement of the Court of Justice of the European Union (CJEU) in Case C-528/16 writes of "mutagenesis techniques which have conventionally been used in a number of applications and have a long safety record". SDN-1 and 2 and ODM do not show additional risks and should be equally evaluated as safe technologies. In fact all GMOs have a long safety record as not a single hospitalization occurred worldwide since when in 1983 the first Gm plant was described in the scientific literature. Even the term "EFSA GMO panel" sounds quite old and the unscientific term GMO should be replaced by a more science-based and updated definition, e.g. "Agri-food biotechnology panel".	The GMO Panel thanks SETA for the comments and takes note of them. The sentence reported in the comment ("mutagenesis techniques which have conventionally been used in a number of applications and have a long safety record") has been provided by the European Commission in the frame of the mandate and it has been reported as it is in this opinion. The GMO Panel was not mandated to express an opinion on how genome edited plants should be	321

			risk assessed but rather to assess the applicability of the section 4 and conclusions of the opinion on SDN-3 to plant obtained via SDN-1, SDN-2, and ODM.	
Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG)	Other comments	The scientists organized in the association "Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG) welcome the opportunity to comment on the draft EFSA opinion "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 type 2 and oligonucleotide-directed mutagenesis. In the following the abbreviation WGG is always used for the association "Wissenschaftlerkreis Grüne Gentechnik e. V.". The WGG refers exclusively to the "Terms of Reference" and makes no comments on what else would be desirable with regard to the overall problems of the use of genome editing techniques. However, the WGG inform EFSA about the proposals that WGG together with AFBV sent to the Commission in February 2020. (Proposal by AFBV and WGG for amendments to GMO legislation and Explanatory Note supporting the AFBV-WGG Initiative - Suggestions to enable the development of genome editing in Europe attached separately) WGG supports the opinion and conclusions of the EFSA GMO Panel that plants whose genetic information has been altered by SDN-1, SDN-2 or ODM pose no fundamental new or additional risks to humans and the environment. There is no evidence of this in the scientific literature. The potential risks do not differ from that resulting from the SDN-3-techniqus, classic breeding or the classical mutagenesis processes. Thus, the safety assessment procedures used so far can be applied. However, a differentiated consideration must be performed as to whether the modified plant contains "foreign" nucleic acids which is often not the case with SDN-1, SDN-2 procedures and ODM. In this case, the corresponding assessment procedures must be adapted or the assessment must be based primarily on the product to be placed on the market. For gene edited plants, which could also result from natural mutations, the mandatory 90-day feeding should be waived (Regulation (EU) No. 503/2013).	The GMO Panel thanks WGG for the comment.	322
Union Française des Semenciers	Other comments	Preliminary remarks: UFS considers that EFSA GMO Panel does not give enough consideration to the principle of proportionality in its evaluation. As set out in Article 5 of the EU Treaty (TEU), the principle of proportionality has been included in the general food law which states "In accordance with the principle of proportionality as set out in Article 5 of the Treaty, this Regulation does not go beyond what is necessary in order to achieve the objectives pursued" (Regulation (EC) No 178/2002). Consequently, this principle must be taken into consideration in the evaluation of every mandate. Accordingly EFSA should ensure that its measures and requests are appropriate and non-discriminatory to achieve the overall objective of safety, without going beyond what is necessary to achieve that objective. UFS would not consider it proportionate when among two identical plants with similar risk profiles, one would be submitted to a stringent risk assessment because of the breeding method used for its development.	The GMO Panel thanks for the comment and takes note of it. The GMO Panel was not mandated to express an opinion on how genome edited plants should be risk assessed but rather to assess the applicability of the section 4 and conclusions of the opinion on SDN-3 to plant obtained via SDN-1, SDN-2, and ODM. The "principle of proportionality" was not addressed in the opinion on SDN-3 and therefore were not specifically addressed in this opinion. However, given the variety of products achievable with genome editing techniques, the	323

			GMO Panel considers that the concept of case-by-case approach, as described in the opinion on SDN-3, is also applicable to genome edited plants.	
Fachstelle Gentechnik und Umwelt	Other comments	Please consider our new publication "Broadening the GMO risk assessment in the EU for genome editing technologies in agriculture", which was recently accepted by the journal Environmental Sciences Europe and is now in press, in the process of your public consultation. We describe the range of specific unintended effects associated with genome editing and examine the considerable possibilities to change the genome of plants and animals with SDN-1 and SDN-2 genome editing (i.e. without the insertion of genes conferring the novel trait) and show that genome editing techniques are able to produce a broad spectrum of novel traits that, thus far, were not possible to be obtained using conventional breeding techniques. We consider that the current EU risk assessment guidance for GMOs requires revision and broadening in order to capture all potential genomic irregularities arising from genome editing and suggest additional tools to assist the risk assessment of genome-edited plants and animals for the environment and food/animal feed in the EU.	The GMO Panel takes note of the comment. To develop the opinion, the GMO panel not only evaluated review and opinion papers but also research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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. In order to clarify its positions, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references.	324
Scientific Committee for GM food and Feed, Advisory Body, Czech Republic	Other comments	Scientific committee for GM food and feed appretiate work of GMO panel under current legal requirements Incomplete vesion was submitted some time ago. on behalf ofr SCFFGMO Jaroslava Ovesná	The GMO Panel thanks for the comment.	325
European Coordination Via Campesina	Other comments	In general, the safety of new genomic techniques such as the SDN techniques has not been evaluated and scientific studies show that these techniques result in unexpected alterations of the genome, both at the intended target and off-target sites. Any of these alterations could result in unexpected toxicity and/or allergenicity. The lack of knowledge also relates to the environmental and cumulative effects that may result from the products of these techniques. The following is an overview of scientific studies on these issues: https://www.gmwatch.org/en/news/latest-news/19223 EFSA assessments should look better into the unintended effects of those techniques, the assessment of the socio-economic, health, and environmental impacts of the marketing of those products intended for agriculture or agro-industry and the assessment of the risks related to the dissemination of those products in terms of biosafety.	The GMO Panel takes note of the comment. For the response to this comment and to all the related aspects, the GMO Panel invites the European Coordination Via Campesina to refer to the specific responses in the other sections of the opinion.	326
German Plant Breeders' Association (BDP -	Other comments	BDP is of the opinion that plants developed through the latest breeding methods should not be subject to different or additional regulations if they could also be obtained through earlier breeding methods or result from spontaneous processes in nature. SDN1/2 and ODM approaches qualify as	The GMO Panel takes note of the comment.	327

Bundesverband Deutscher Pflanzenzuechter e.V.)		leading to such genetic alteration in plants that should not require different or additional regulation as long as resulting plants do solely contain genetic material from sexually compatible species.		
COST Action CA18111 - Plant genome editing – a technology with transformative potential (PlantEd)	Other comments	This opinion addresses specific sections of EFSA risk assessment guidance on molecular characterization and confirms convincingly that SDN-1, SDN-2 and ODM based directed mutagenesis is similar to and undistinguishable from mutations obtained by conventional breeding techniques, and that their application entail no new specific hazards. While acknowledging the suggested and scientifically justified reduction in molecular data requirements, there is no clarity on other data requirements. PlantEd invites EFSA to present a clear problem formulation that spells out which of the requirements for risk assessment of GM plants, or food and feed products resulting from those plants, are essential in order to achieve a high level of safety for European consumers, animals and the environment. This level of safety should be comparable to the level for conventionally bred crops. This would help aligning the EU GMO risk assessment with the principle of proportionality.	The GMO Panel thanks for the comment. The GMO Panel was not mandated to express an opinion on how genome edited plants should be risk assessed but rather to assess the applicability of the section 4 and conclusions of the opinion on SDN-3 to plant obtained via SDN-1, SDN-2, and ODM. For this reason, a complete list of all the requirements not applicable for the risk assessment of genome edited plants is not provide in this opinion. Nevertheless, given the variety of products achievable with genome editing techniques, the GMO Panel considers that the case-by-case approach, as described in the opinion on SDN-3, is also applicable to genome edited plants.	328
French agency for Food, Environmental and Occupational Health & Safety (Anses)	Other comments	General comment #1: Overall, the document is well written and of good scientific quality. However, the way the off-target effects were considered is a major concern for Anses and several comments are made on this topic. General comment #2: Although it is claimed that the CRISPR/Cas9 gene editing system does not include unwanted mutations at off-target sites, our understanding of the Cas9 specificity still remains limited with respect to Cas9-edited plants. Even though no off-target mutations were detected in cotton (Zhu et al., 2018) and maize (Feng et al., 2018), rare off-target mutations could be detected in soybean (al Amin et al., 2019). In addition to off-target mutations, endonuclease Cas12a, used in the bacterial CRISPR-Cas12a system, exhibited multiple nicking activities against the dsDNA substrate (Murugan et al., 2020). With respect to these changes, whole genome sequencing should be a valuable tool for revealing unwanted off-target mutations resulting from the use of CRISPR/Cas gene editing systems in plants (Li et al., 2019). However, despite the difficulty to prevent unwanted mutations to be generated, different approaches can be used to improve the edition efficiency like the association of guide RNAs (gRNAs) to the Cas9 nuclease (Charrier et al., 2019) and the use of ligand-dependent ribozymes aptazymes associated to	Regarding comment #1, the GMO Panel thanks for the comment. The GMO Panel invites ANSES to refer to the related responses given for the off-targets related comments in the specific section of the opinion. Regarding comment #2, the GMO Panel takes note of the comment.	329

		guide RNAs (gRNA), to reduce the frequency of off-target mutations in plants (Hajiahmadi et al., 2019). Recently (Qin et al., 2020), the off-target risk associated to the self-target effect of the Streptococcus pyogenes SpCas9-NG, susceptible to generate new single-guide RNAs, could be alleviated by using a modified single-guide RNA scaffold starting with the GCCCC sequence stretch. The use of endonuclease Cas12a in the bacterial CRISPR-Cas12a system should be avoided, due to its multiple nicking activities on the dsDNA (Murugan et al., 2020). References: Zhu, S., Yu, X., Li, Y., Sun, Y., Zhu, Q., Sun, J. Highly efficient targeted gene editing in upland cotton using the CRISPR/Cas9 system. Int. J. Mol. Sci. 2018;19:3000. Feng, C., Su, H., Bai, H., Wang, R., Liu, Y., Guo, X., Liu, C., Zhang, J., Yuan, J., Birchler, J.A., Han, F. High-efficiency genome editing using a dmc1 promoter-controlled CRISPR/Ca9 system in maize. Plant Biotechnol. J. 2018;16:1848-1857. al Amin, N., Ahmad, N., Wu, N., Pu, X., Ma, T., Du, Y., Bo, X., Wang, N., Sharif, R., Wang, P. CRISPR-Cas9 mediated targeted disruption of FAD2-2 microsomal Ω-6 desaturase in soybean (Glycine max L.). BMC Biotechnol. 2019;19:9. Murugan, K., Seetharam, A.S., Severin, A.J., Sashital, D.G. CRISPR-Cas12a has widespread of target and dsDNA-nicking effects. J. Biol. Chem. 2020;295:5538-5553. Li, J., Manghwar, H., Sun, L., Wang, P., Wang, G., Sheng, H., Zhang, J., Liu, H., Qin, L., Rui, H., Li, B., Lindsey, K., Daniel, H., Jin, S., Zhang, X. Whole genome sequencing reveals rare off-target mutations and considerable inherent genetic or/and somaclonal variations in CRISPR/Cas9-edited cotton plants. Plant Biotechnol. J. 2019;17:858-868. Charrier, A., Vergne, E., Dousset, N., Richer, A., Petiteau, A., Chevreau, E. Efficient targeted mutagenesis in apple and first time edition of pear using the CRISPR-Cas9 system. Front. Plant Sci. 2019;10:40. Hajiahmadi, Z., Povahedi, A., Wei, H., Li, D., Orooji, Y., Ruan, H., Zhuge, Q. Strategies to increase on-target and reduce off-target effects of the CRISPR/Cas9 system in plants. Int. J. Mol. Sci. 2019;20:3719. Qin, R., Li, J., Liu, X., Xu, R., Yang, J., Wei, P. SpCas9-NG self-targets the sgRNA sequence in plant genome editing. Nat. Plants 2020;6:197-201.		
Corteva Agriscience	Other comments	Terms "gene editing" and "genome editing" are interchanging throughout the document.	The terms "gene editing" in the title of section 3.1.1 has been changed to "genome editing".	330
European Plant Science Organisation, EPSO	Other comments	EPSO considers that the recently drafted EFSA opinion evaluates its preceding opinion on the risk assessment of site-directed nucleases type 3 published in 2012 in a balanced manner with regard to the transfer of relevant parts to the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide-directed mutagenesis. EFSA explored and pointed out the limits and possibilities to adopt the risk assessment approach for SDN-3 to applications of SDN-1,	The GMO Panel thanks for the comment and invites EPSO to refer to the related responses given to comments in the respective sections of the opinion.	331

		SDN-2 and ODM. Essentially EFSA promotes a case by case risk evaluation and outlines possible simplifications with regard to specific genome editing methods. Nevertheless, EPSO would like to raise a few issues to achieve more clarity and consistency in some parts of the drafted document (see comments to chapters inserted before).		
Testbiotech	Other comments	There are currently two EFSA drafts under discussion that deal with SDN-1 and SDN-2 plants. These draft documents vary widely in findings and conclusions, without giving any explanation or reasoning. To streamline these drafts, we recommend an additional text as an introduction, taking into account some findings from the Report SCENIHR, SCHER and SCCS (2015) on Synthetic Biology II: "Genome editing, using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system, Transcription activator-like effector nucleases (TALENs) or zinc-finger nucleases (ZNF) enables the rapid introduction of targeted genetic modifications in existing genomes. These techniques can be applied in a wide range of higher organisms (plants, animals), accelerating their genetic modification considerably (from many months to a few weeks in the case of mice) and facilitating the modification of non-model organisms. A large number of modifications may be introduced in parallel. New techniques may also be used in a multiplexed fashion, allowing the simultaneous generation of large numbers of variants that can then be screened or selected for desired properties. This will create additional challenges from a risk assessment standpoint, in that organisms produced by these methods may contain more pervasive changes to the genomes of living organisms than previous genomic techniques."	The GMO Panel thanks Testbiotech for the comment and takes note of it. Please note that a parallel opinion on synthetic biology is being prepared where the aspects raised in the comment are discussed.	332
Umweltbundesamt (Environment Agency Austria) on behalf of the Austrian lead Competent Authority, the Federal Ministry of Social Affairs, Health, Care and Consumer Protection.	Other comments	General Comments: As a general observation we are of the opinion that the draft opinion at hand does not adequately address the subject matter of describing appropriate risk assessment approaches for plants developed using site-directed nucleases type 1 and 2 and oligonucleotide-directed mutagenesis. This may be partly due to the the terms of reference (ToR) provided by the European Commission calling for an examination of the applicability of the EFSA Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function (EFSA 2012a) instead of asking to provide a guidance for risk assessment addressing the generic issues associated with SDN-1, SDN-2 and ODM applications. The opinion is a maze of generalized references and cross-references to previous opinions such as EFSA (2012a) and EFSA (2012b) as well as other guidance documents (e.g. EFSA 2010, 2011) and therefore cannot be used as a stand-alone document addressing new mutagenesis techniques. Specifically the draft opinion fails to provide an appropriate case-specific discussion of the various different SDN-based techniques and outcomes, which should be addressed by specific and focused risk assessment approaches. The discussion of a case-specific approach should be based on an analysis of (available) examples to better illustrate the specific requirements and recommendations to address the respective risk issues. In addition we want to share some other general observations concerning the draft document:	The GMO Panel thanks for the comment. As correctly highlighted in the comment, the GMO Panel was not mandated to express an opinion on how genome edited plants should be risk assessed but rather to assess the applicability of the section 4 and conclusions of the opinion on SDN-3 to plant obtained via SDN-1, SDN-2, and ODM. Following the public consultation, the text of the opinion has been revised to improve clarity and consistency.	333

		Firstly the document is presented in a highly elaborated structure, yet it contains limited substantive information. We recommend that the focus of the revision is laid on providing additional specific information addressing case-specific considerations. Secondly the revision should include a review of the used terminology to avoid misunderstandings and to increase the consistency of the use of relevant terms such as "risk assessment" vs. "safety assessment" , "technology" vs. "technique", "new mutagenesis techniques", etc. EFSA (2012a): EFSA Panel on Genetically modified organisms (GMO); Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function. EFSA Journal 2012;10(10):2943. EFSA (2012b): EFSA Panel on Genetically Modified Organisms (GMO); Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragen-esis. EFSA Journal 2012;10(2):2561. l EFSA (2011): EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011; 9(5): 2150. EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Guidance on the 491 environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879.		
Sciensano	Other comments	-What is the added-value from a safety perspective of submitting plants developed by SDN-1, -2 and ODM to a risk assessment under the GMO regulatory framework, while such a risk assessment is not requested for plants obtained through classical mutagenesis? - Does Article 5.2 of Regulation 503/2013 (see below) apply to plants developed by SDN-1, -2 and ODM so as to permit notification of such plant products with a limited set of data? If yes, what information would be covered by the derogation? Article 5: 1. Information, including studies, required to accompany the application as referred to in Article 5(3)(a) to (f) and (h) and in Article 17(3)(a) to (f) and (h) of Regulation (EC) No 1829/2003 shall be provided in accordance with the scientific requirements for the risk assessment of genetically modified food and feed set out in Annex II to this Regulation. 2. By way of derogation from paragraph 1, an application may be submitted that does not satisfy all the requirements of that paragraph provided that: (a) particular information is not necessary owing to the nature of the genetic modification or of the product; or (b) it is not scientifically necessary, or technically possible to supply such information. The applicant shall submit reasoned justification for the derogation.	The GMO Panel was not mandated to express an opinion on whether or not genome edited plants should be risk assessed but rather to assess the applicability of the section 4 and conclusions of the opinion on SDN-3 to plant obtained via SDN-1, SDN-2, and ODM. Given the variety of products achievable with genome editing techniques, the GMO Panel considers that the case-by-case approach, as described in the opinion on SDN-3, is also applicable to genome edited plants. In this respective, the GMO Panel concluded that a number of requirements of the existing guidances that are linked to the presence of a transgene are not relevant for the risk assessment of SDN-1, SDN-2 and ODM plants in case the final product does not contain any exogenous DNA.	334

Società Italiana di Genetica Agraria - Italian Society of Agricultural Genetics (SIGA)	Other comments	GENERAL COMMENT SIGA welcomes the EFSA Scientific Opinion Draft "Applicability of the EFSA opinion on site-directed nucleases type3 for the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide-directed mutagenesis". In the past few years SIGA and other major Italian plant science and agricultural science societies have expressed similar positions regarding SDN-1 and SDN-2 (an extensive version of our position and indications can be found here: http://www.geneticagraria.it/attachment/SocietaScuolaRicerca/NBT_SIGA-SIBV_en.pdf). The basic principle that a new plant variety should be considered for what it is and not according to the technique used to produce it is the only rational approach to risk assessment. Consequently, a revision of Directive 2001/18/EC is deemed necessary. With this in mind, it would be very useful to make a more specific statement on the extent of SDN-2-based modifications for which no specific risk assessment beyond that operating for conventional breeding should be necessary. We refer to the introduction of modifications that reproduce alleles of genes that already exist in the same species or in its sexually compatible relatives. The introduction of such mutations by SDN-2 represents the most precise technology – and the one that involves the lowest risk - when the aim is to introduce useful traits between organisms that can be interbred. The Italian Society of Agricultural Genetics (Società Italiana di Genetica Agraria, SIGA, www.geneticagraria.it/) is a Scientific Society founded in 1954. Its mission is fostering research and education in the fields of agricultural genetics, genomics, breeding and biotechnology of agricultural organisms. SIGA has about 300 members, from Universities, public research institutes and from the private sector.	The GMO Panel thanks SIGA for the comment. The GMO Panel was not mandated to express an opinion on whether or not genome edited plants should be risk assessed but rather to assess the applicability of the section 4 and conclusions of the opinion on SDN-3 to plant obtained via SDN-1, SDN-2, and ODM. Specifically, defining the extent of the SDN-2 modification that would require the product to be risk assessed was not in the remit of this mandate.	335
Ministry of Agriculture, Livestock and Food Supply of Brazil	Other comments	The introduction of variability in species of economic importance can help achieve important sustainability goals such as the production of healthier foods, less use of agricultural chemical pesticides and preservation of the environment. Innovations in the field of genetics must be made respecting the basic principles of biosafety, however, legislation cannot stop technological development. If products resulting from targeted mutagenesis, mainly SDN1 and SDN2 systems are considered to have the same risk assessment requirements as traditional GMOs / SDN3, it may restrict technological development to a few and larger companies, limiting market competition, as well as access to benefits from generating products with these technologies. In alignment with the international scientific community and the legislation of several countries, it is understood the importance of harmonization in the biosafety laws of food exporting and importing countries that reflects and welcomes technological progress, maintaining the quality and safety of food, but that also allows a greater diversification of the participants in the production chain. Products generated by genome editing, mainly SDN1 and SDN2 systems, should not be subject to risk analysis requirements similar to what is done with GMOs if they can also be obtained by conventional methods or result from spontaneous processes in nature. Thus, bearing in mind that gene editing techniques require less data for analysis, considering that the modification is, in many cases, similar to conventional breeding and that there is no introduction of a transgene ("Indeed, as SDN-1, SDN-2 and ODM aim at modifying endogenous DNA sequence (s), a	The GMO Panel thanks for the comment. The GMO Panel was not mandated to express an opinion on how genome edited plants should be risk assessed but rather to assess the applicability of the section 4 and conclusions of the opinion on SDN-3 to plant obtained via SDN-1, SDN-2, and ODM. For this reason, a complete list of all the requirements not applicable for the risk assessment of genome edited plants is not provide in this opinion. Nevertheless, given the variety of products achievable with genome editing techniques, the GMO Panel considers that the case-by-case approach, as described in the opinion on SDN-3, is also	336

		number of requirements of the existing guidances that are linked to the presence of a transgene are not relevant for the assessment of SDN-1, SDN-2 and ODM plants in case the final product does not contain any exogenous DNA."), we understand that it would be useful for EFSA to specifically present the exempting requirements in the current guides in the case of SDN-1 and SDN-2 and ODM, that do not have the transgene. There is a description in the text about the need for a smaller amount of experimental data, the ineffectiveness of analyzing off-target potentials, the need to prove the absence of exogenous DNA, but these are very general considerations and a more detailed indication of exempting requirements for a product obtained with SDN-1, SDN-2 and ODM would be useful.	applicable to genome edited plants.	
Cornell University's Alliance for Science	Other comments	EFSA's decision on the risk assessment of SDN1, SDN2, and ODM produced plants will have far reaching effects on the utilization of gene editing in agriculture in Europe and elsewhere in the world. Applying different risk assessment requirements to SDN-1, SDN-2, and ODM produced plants based on the final product obtained would allow EFSA to address risks proportionately on a case-by-case basis. This approach would encourage European scientist and breeders to safely use genome-editing techniques to produce crops that have sustainability, bio-fortification and other consumer beneficial traits that can reach farmers without an expensive and lengthy regulatory process. As stated recently by the EU commission, "New innovative techniques, including biotechnology and the development of bio-based products, may play a role in increasing sustainability, provided they are safe for consumers and the environment while bringing benefits for society as a whole. They can also accelerate the process of reducing dependency on pesticides." How EFSA determines the safety of SDN-1, SDN-2, and ODM produced plants will have impacts beyond its geographic borders. Africa is monitoring how Europe's regulators handle Gene Editing (GE) techniques and EFSA's decisions on how it regulates those crops could impact that continent's adoption of gene-edited products useful to its farmers. A tiered, risk-based regulatory approach that reflects science-based assessments will encourage adoption of similar regulatory systems by African countries. This would benefit on-going GE programs like the development of disease-resistant crops in Kenya and make it feasible for development and adoption of publicly-funded, R&D that can help African countries achieve a number of the Sustainable Development Goals as defined by the UN. However, if the EU adopts a overly precautionary regulatory approach, the concern about losing potential export markets could lead to genome-edited plants not being used in African countries at all, even though they could be particularly beneficial.	The GMO Panel thanks for the comment and takes note of it.	337
GenØk-centre for biosafety	Other comments	For details regarding this section: please read our attached table with our comments, also for section 3.3 regarding ToR2 of the mandate and suggested table called ANNEX I with supporting techniques for the distinct SDN teqhniques. The distinct Cas proteins used in the Crispr/cas technology, are known to have off-target activity, affected by different factors like:concentration, type of protein etc and is a driving force for the ongoing development of more effective and specific Cas proteins. This is not discussed in the draft as an issue. Also, the issue of using plasmids in TALEN edition is important as it has been found that parts of plasmids have ended up, being integrated in genomes of living organisms, like the hornless cows where abr genes ended up in the genomes, active. There should therefore be a higher focus of analysis of off-target effects and the potential changes these can bring to cells and how these can affect other mechanisms inside cells and also how to minimize this effects.Specificity, even with Crispr/cas techniques have been, and still is an ongoing focus.	The GMO Panel thanks for the comment. To develop the opinion, the GMO panel took into consideration research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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.	338

			Nevertheless, the GMO Panel has revised the text of the opinion, accordingly, including some additional relevant references in order to clarify its positions. Regarding the unwanted integration of exogenous DNA (for example, plasmid or DNA fragments), a specific section is already included in chapter 3.2.2.2.2 which deals with alteration outside the target site.	
Federal Agency for Nature Conservation	Other comments	The draft focuses on the questions whether SDN-1, SDN-2 and ODM interventions – like SDN-3 – insert foreign genes or not and on intended changes at the target site. However, it disregards the present and upcoming potentials and possibilities especially of SDN-1 and SDN-2 for deep genomic interventions (see comment on 3.1.1), the possibility for unintended changes at and around the target site (see comments 3.2.2.2.1) and possibly changes due to several steps involved in SDN interventions (see comment on 3.). Also, the draft disregards that conventional breeding and genome editing take two distinct approaches to achieve a new trait (see comment on 2.1.3). Because of this narrow approach, we do not share the overall conclusion that no new and specific hazards specifically linked to the genomic modification produced via SDN-1, SDN-2 and ODM as compared to both SDN-3 and conventional breeding were identified, but advocate to assess the entire plant (see comment lines 186-273.	The GMO Panel thanks for the comment and invites the contributor to refer to the responses given to the specific comments for line 186-273 of the opinion.	339
GMWatch	Other comments	In this draft document, EFSA makes many assumptions that are not supported by scientific evidence and that are in many cases directly contradicted by scientific evidence. EFSA appears to be unaware of a large body of peer-reviewed scientific studies drawing attention to the imprecise nature of gene editing involving unintended on-target and off-target effects, as well as the risks to food safety and the environment that are posed by novel crops developed using these techniques. We draw attention to some of these studies in this document but emphasise that our list is only partial. It is EFSA's responsibility to conduct a literature survey for relevant studies and directly address the risks that they raise. Many of these studies were conducted in animals or human or animal cells, since researchers in the medical field have been more proactive in researching the risks and unintended effects of gene editing than researchers in the plant biotechnology field, who focus more on product development. However, the same issues of unintended on-target and off-target effects also apply to plant gene editing. Exactly what effects these errors might have on food safety and environment are still unknown, as no one has looked into this, but existing evidence (summarised below) is suggestive. It is EFSA's responsibility to state that these risks exist and to clarify that their full implications are not yet understood, rather than to minimize and dismiss the risks, as in this draft document. Unintended effects in gene-edited plants will alter patterns of gene function and thus carry the risk of changing the plant's biochemistry, which can lead to production of unexpected toxins or allergens. Molecular profiling (proteomics, metabolomics) compositional analysis and controlled laboratory animal feeding studies show that these problems have arisen by the same basic mechanism of unexpected alterations in the global patterns of gene expression with the first generation of GM crops	The GMO Panel was not mandated to deliver a comprehensive literature review on genome editing in plants but rather to assess the applicability of the section 4 and conclusions of the opinion on SDN-3 to plant obtained via SDN-1, SDN-2, and ODM. In section 3.2.2, the GMO Panel acknowledges that SDN-based techniques can introduce off-target mutations. However, the GMO panel took also into consideration research papers that provided actual experimental data on off-target mutations and their analysis. These papers present 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	340

		(see: Mesnage M et al (2016). An integrated multi-omics analysis of the NK603 Roundup-tolerant GM maize reveals metabolism disturbances caused by the transformation process. Scientific Reports 6. http://www.nature.com/srep/2016/161219/srep37855/full/srep37855.html); also reviews: Krimsky S (2015). An illusory consensus behind GMO health assessment. Science, Technology & Human Values. http://sth.sagepub.com/content/early/2015/08/05/0162243915598381; Hilbeck A et al (2015). No scientific consensus on GMO safety. Env Sci Europe 27(1):4. http://www.enveurope.com/content/27/1/4/abstract). Despite the above observations no animal feeding trials have been carried out with new gene-edited plants, so claims of food safety are based on assumptions and not on experimental evidence. The draft document – and EFSA's 2012 Opinion on the risk assessment of plants developed with SDN-3, which the document references – omits a large number of studies and reviews that have been published since 2012 (see "List of studies" in the attachment to this submission) that draw attention to unintended on-target and off-target effects from gene editing (most of them involve SDN-1 and SDN-2 techniques, though no. 7, Norris AL et al (2020), is SDN-3). It is not valid to dismiss studies in human or animal cells or in living animals as not relevant to plants. Just one example of a study in animals that has clear relevance to plants is study no. 8 above, Skryabin BV et al. (2020) (https://advances.sciencemag.org/content/6/7/eaax2941), which showed that when the CRISPR/Cas system was used in an SDN-2 gene-editing procedure aimed at engineering the insertion of genetic material in mice, a high frequency was found of insertions of multiple copies of the DNA molecules used as a template for bringing about the desired gene modifications. The researchers were concerned by the fact that the insertions could not be detected using standard PCR analysis. This led to what they called "a high rate of falsely claimed precisely edited alleles" (gene variants). The researchers used an extended PCR analytical method and found that in most cases, there were multiple head-to-tail insertions of the template repair DNA molecule. The authors themselves drew attention (https://www.the-scientist.com/news-opinion/crispr-can-create-unwanted-duplications-during-knock-ins-67126) to the fact that their findings have relevance for gene editing across all kingdoms of life, from human cells to plants. They warned that duplications could lead to dangerous frameshift mutations, resulting in misshapen proteins. The conclusions regarding potential allergenicity and/or toxicity should be clear. In the draft document, EFSA has omitted to address the issue of unintended on-target effects of gene editing, which are demonstrated in some of the above-cited studies.	breeding including random mutagenesis. Based on this, the section has been revised to clarify the position of the GMO Panel on off-target effects.	
BUND e.V. / Friends of the Earth Germany	Other comments	There are risks not being addressed at all in the opinion so far. As added inside the chapters, important findings on on- and off-target-effects have not been considered for this opinion at present stage. Further risks such as those linked to the use of multiplexing – parallel knock-out/alteration of more than one gene – are not being addressed yet, but need to be considered, as unintended effects, including pleiotropic effects, may show up (Li et al. 2017). The question of environmental risks is covered insuffieiently too, as the fitness of genetically modified	The GMO Panel takes note of the comments. Please refer to section 3.2.2.2 and the related comments and responses for all the considerations related to off-targets. The GMO Panel concluded that the ERA EFSA guidance (EFSA GMO Panel 2010) is applicable. It should be noted that multigene modifications leading to the	341

		plants and their interactions with their microbiome, soil life, arthropods and other animals may change. For instance, a reduced amount of linolenic acid (as achieved in Calyno soybean produced by genome editing) may lead to a reduced amount of jasmonic acid, which then could reduce resistance of plants to biotic and abiotic stress (Wang et al. 2020). This leads to the conclusion that environmental risk assessment has to be extended in general, but the question of environmental risks must become part of this opinion as well. Interesting enough the EFSA – opinion´s draft on environmental risk assessment of genetically modified plants obtained through synthetic biology published at the same time draws conclusions with regard to potentially additional challenges to risk assessement that vary from those in the present opinion. For this reason, it seems to be appropriate all the more to properly address potential environmental risks, including those specific to SDN-1 and SDN-2 applications and those that may be higher in applying new techniques such as CRISPR/Cas then applying previous breeding techniques.	alteration of existing traits or the generation of new complex ones, including e.g. modification of plant metabolism affecting multiple signaling pathways, could also be achieved by conventional breeding and traditional transgenesis; hence, this is neither a novel scenario nor a new hazard which is limited only to genome edited plants. Moreover, compositional and phenotypical analysis of genome edited plants is still a pillar of the risk assessment under the current EU regulation of GMOs. The GMO Panel would also like to remind that the "case-by-case" approach as described in the opinion on SDN 3 is also applicable to genome edited plants.	

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

EFSA's GMO Panel has launched an open consultation on its draft scientific opinion on the applicability of the EFSA opinion on site-directed nucleases type 3 (SDN-3) for the safety assessment of plants developed using site-directed nucleases type 1 and 2 (SDN-1 and SDN-2) and oligonucleotide-directed mutagenesis (ODM). In line with the mandate of the European Commission, this opinion takes into consideration section 4 and conclusions of the EFSA opinion on SDN-3 (EFSA GMO Panel, 2012) and evaluates the applicability of those sections for the risk assessment of plants produced via SDN-2, SDN-3, and ODM approaches.

Interested parties are invited to submit written comments by **27 May 2020**. Please use the electronic template provided [https://ec.europa.eu/eusurvey/runner/PC_SDN_Site-Directed_Nucleases] to submit comments and refer to the line and page numbers. Kindly note that after 2 hours of non-activity your working session will expire, and comments submitted after that time will not be recorded and transmitted. Therefore, if the page is left inactive for more than 2 hours, please re-open it from the link before restarting to comment. If you would like to submit additional data to support your comments or file, send an email to: GMOManagement@efsa.europa.eu.

Please note that comments will not be considered if they:

- are submitted after the closing date of the consultation;
- are presented in any form other than what is provided for in the instructions and template;
- are not related to the contents of the document;
- contain complaints against institutions, personal accusations, irrelevant or offensive statements or material;
- are related to policy or risk management aspects, which is out of the scope of EFSA's activity.

EFSA will assess all comments from interested parties that are submitted in line with the criteria above. The comments will be further considered by EFSA's GMO Panel and taken into consideration if found relevant.

Copyright-cleared contributions

Persons or organisations participating in a public consultation of EFSA are responsible for ensuring that they hold all the rights necessary for their submissions and consequent publication by EFSA. Comments should *inter alia* be copyright-cleared taking into account 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

Contributions will be published (as part of an EFSA report published together with the final scientific opinion) and may be re-used by EFSA in a different context. It should be noted that contributions submitted by individuals in a personal capacity will be published as such, indicating the author's first and family name, unless a substantial justification for protection is provided by the respondent. Contributions submitted on behalf of an organisation are also made publicly available and attributed to the organisation in question.

Submit comments (deadline: **27 May 2020**)

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Abbreviations

Cas9	CRISPR associated protein 9
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DSB	Double strand break
EC	European Commission
EFSA	European Food Safety Agency
ERA	Environmental Risk Assessment
EU	European Union
GM	Genetic Modification / Genetically Modified
GMO	Genetically Modified Organism
MC	Molecular Characterization
NGO	Non-Governmental Organization
NHEJ	Non-homologous end joining
ODM	Oligonucleotide-Directed Mutagenesis
RA	Risk Assessment
SDN	Site-Directed Nuclease
sgRNA	Single guide RNA
ToR	Terms of Reference
WG	Working Group

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