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Public consultation on the draft scientific opinion on the evaluation of existing guidelines for their adequacy for the food and feed risk assessment of plants obtained through synthetic biology

European Food Safety Authority (EFSA)

Abstract

In line with the European Food Safety Authority's (EFSA) 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, EFSA carried out a public consultation to receive input from interested parties on the draft scientific opinion on the evaluation of existing guidelines for their adequacy for the food and feed risk assessment of plants obtained through synthetic biology. This draft scientific opinion was prepared by the EFSA GMO Panel, supported by a Working Group on Synthetic Biology of Genetically Modified Plants. The draft opinion was endorsed by the EFSA GMO Panel for public consultation on 1 December 2021. The online public consultation was open from 19 January 2022 until 20 March 2022 by means of an electronical comment submission tool together with explanatory text on the EFSA website (See Appendix A). EFSA received comments from 8 different interested parties. EFSA and its GMO Panel wish to thank all stakeholders for their contributions to this work. The present Annex 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 20 June 2022 and will be published in the EFSA Journal.

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1. Introduction

Table 1 provides an overview on the organisations that have submitted comments through the electronic online tool (CropLife Europe, German Central Committee on Biological Safety ZKBS, BfN and Testbiotech uploaded additional files in the online tool, see Appendix B.).

The comments received were duly evaluated by the EFSA Working Group of the GMO Panel on Synthetic Biology of Genetically Modified Plants. Wherever appropriate these comments were taken into account for finalisation of the draft opinion on the evaluation of existing guidelines for their adequacy for the food and feed risk assessment of plants obtained through synthetic biology.

Table 2 provides a detailed list with all comments received from organisations 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 1: Overview on stakeholder comments received

Organisation Name[(a)]	**Country**
ANSES	France
BfN - Federal Agency for Nature Conservation	Germany
Croplife Europe	Belgium
Euroseeds	Belgium
Federal Office of Consumer Protection and Food Safety (BVL) (National Authority)	Germany
German Central Committee on Biological Safety (ZKBS)	Germany
National Food Institute, Technical University of Denmark	Denmark
Testbiotech	Germany

(a): As specified by the commenter.

2. Comments received

See Table 2.

Table 2: Comments and responses from EFSA

No	Section	Organisation Name	Comments	Responses from EFSA
1	1. Introduction	Federal Office of Consumer Protection and Food Safety (BVL)	Does "engineered organisms" mean "genetically modified organisms" in this context? If so, it is recommended to use the full term "genetically modified organisms". If not the meaning of the term "engineered organisms" needs to be specified and an explanation given why it is used.	In the context noted by the commenter the term "engineered organisms" means "genetically modified organisms". The text of the scientific opinion has been amended accordingly.
2	1.Introduction	ANSES	GMOs guidelines should be revisited on a regular basis taking into account new technologies. Three previous Opinions (SCENIHR, SCCS, and SCHER, 2014, 2015a,b) on SynBio concluded that new SynBio applications may be assessed using current risk assessment methodology for genetically modified organisms (GMOs))	Comments noted. It is highlighted that EFSA is actively following scientific and technological developments as demonstrated by numerous activities undertaken in the last decade. These range from the organisation of workshops and scientific colloquia to promoting procurements and grants addressing development projects. The applied risk assessment approaches for GMPs are regularly updated by EFSA based on scientific and technological developments, as well as regulatory developments. This can result in guidance document update, specific notes to the guidance documents or publication of new risk assessment strategies of the GMO Panel in the notes of the GMO Plenary. A non-comprehensive overview of the recent activities is available in Section 3.6 of EFSA, 2020. As regards specifically New Genomic Techniques, since 2019 EFSA is addressing several mandates from the European Commission that may have implications for future applications for GMO authorisation on the EU market and their risk assessment (e.g. EFSA GMO Panel, 2020). Regarding the comment that new SynBio applications may be assessed using current risk assessment methodology for genetically modified organisms, the Convention on Biological Diversity further clarified that 'While there is no internationally agreed definition of 'synthetic biology', key features of synthetic biology include the de novo synthesis of genetic material and an engineering-based approach to develop components, organisms and products.' This further clarification establishes the link for the request to support the EU in the work under the Convention on Biological Diversity and the Cartagena Protocol on Biosafety (2000/2003) (see Section 1.1 of the current scientific Opinion, of EFSA GMO Panel, 2021a and EFSA Scientific Committee, 2020).

3	1.Introduction	CropLife Europe	Line 4: Insert: "rather it is a" OR "rather, it may be considered as a" Line 15: replace "Europe" by "EU". This distinction needs to be ensured throughout the entire document considering other countries in Europe (e.g. the United Kingdom) are developing separate frameworks, thereby deviating from the EU.	Comments noted, text edited.
4	1.Introduction	Euroseeds	Euroseeds welcomes the opportunity to comment on the EFSA draft opinion regarding the Evaluation of existing guidelines for their adequacy for the food and feed risk assessment of genetically modified plants obtained through synthetic biology ("SynBio").	Comment noted.
5	1.1. Definitions for SynBio for the Terms of Reference	ANSES	No comment	Comment noted.
6	1.1. Definitions for SynBio for the Terms of Reference	CropLife Europe	Lines 51-53: The reference made to the Convention on Biological Diversity (CBD) should be clarified to reflect that this is not an official CBD position but a summary prepared by the CBD Secretariat capturing the divergent views of different Parties and other stakeholders on what are the key features of synthetic biology. We recommend the following edit is made to the text to reflect correctly the nature of the information source: In the discussions on synthetic biology under the Convention of Biological Diversity, it has been noted that "While there is no internationally agreed definition of "synthetic biology", key features of synthetic biology include the de novo synthesis of genetic material and an engineering-based approach to develop components, organisms and products."	Comment noted.
7	1.1. Definitions for SynBio for the Terms of Reference	Euroseeds	Lines 51-53: Euroseeds notes that despite the fact that SynBio has been previously defined by the EU Scientific Committees upon request of the EC, there is no internationally accepted definition of "synthetic biology". This is also acknowledged by EFSA. SynBio is used as an umbrella term in the ongoing discussions on CBD level to capture "new" biotechnologies and "new" applications of established biotechnologies, whether actual or conceptual. This might easily lead to a patchwork of regulatory approaches towards SynBio organisms. We recommend the following edit is made to the text to reflect correctly the nature of the information source: In the discussions on	Comments noted. Regarding the comment of the definition of Synthetic Biology, the GMO Panel is aware that there is no internationally accepted definition of Synthetic Biology, however notes that an operational definition is available at EU level, it has been defined in the EC mandate to EFSA and it has been consistently used in the previous works on Synthetic biology by EFSA (EFSA Scientific Committee, 2020 and EFSA GMO Panel, 2021a). See also comment 6. Regarding the case-by-case approach, this is recognized as one of the pillars of the risk assessment of GM plants and derived food and feed: depending on the type of genetic modifications, the outcome of hazard and exposure assessment a tailored approach

			synthetic biology under the Convention of Biological Diversity, it has been noted that "While there is no internationally agreed definition of "synthetic biology" key features of synthetic biology include the de novo synthesis of genetic material and an engineering-based approach to develop components, organisms and products." Euroseeds notes that the case studies considered representative of "synthetic biology" GMPs in the EFSA draft opinion, closely resemble GMPs achieved using "existing transgenic and non-transgenic GMO technologies". Euroseeds therefore agrees with the conclusions of the GMO Panel that had not identified novel potential risks in terms of the impact of SynBio GMPs on humans, animals and the environment, and no novel hazards. In line with the above, we encourage EFSA to further advance the consideration of derogations from certain data requirement outlined in the Implement Regulation 503/2013 that may not be relevant even for transgenic plants. Depending on the product, certain requirements may not be needed or may need to be adapted, consistent with the fundamental principle of a case-by-case approach and fit for purpose risk assessment.	is followed (see EFSA GMO Panel, 2011a and Reg (EU) No 503/2013). The GMO Panel agrees that also for plants developed using synthetic biology approaches the case-by-case approach is essential. The GMO Panel has reminded the importance of this concept in all the recent scientific opinions concerning the techniques that can be used in synthetic biology approaches, such as targeted mutagenesis and transgenesis (EFSA GMO Panel, 2020; EFSA GMO Panel 2021a).
8	1.1. Definitions for SynBio for the Terms of Reference	Testbiotech	Clearly defined language should be used throughout the whole opinion. However, wording used such as "conventional GMP?" or "traditional NGT" are irritating and misleading. Plants derived from genomic techniques which are subjected to EU mandatory approval processes should not be mixed up with plants derived from conventional breeding. Therefore, passages such as line 253 or line 456 need to be revised. Furthermore, it looks like the definition of SynBio plants is mostly based on intended effects and does not allow to clearly categorize or differentiate applications of new genomic techniques (NGT) in general. Therefore, the definition should be revised to be sure that the specific unintended effects caused by the new genomic techniques (NGT) and all relevant organisms derived from NGT are included.	Consistently with the previous opinion on Synbio GMPs (EFSA GMO Panel, 2021a), the term "conventional GMPs" as used in this opinion indicates GMPs obtained through established techniques of genetic modifications. The term "traditional GMP" noted by the commenter in the scientific opinion has been substituted by "GMP applications to date" (see Section 3.2.2). The term "traditional NGT" is not used in the scientific opinion. Regarding the definition of Synbio plants, the GMO Panel used the EU operational definition of Synthetic Biology, see Section 1.1 of the scientific opinion and previous works on the topic (EFSA GMO Panel, 2021a and EFSA Scientific Committee, 2020). Accordingly, SynBio is a process or strategy comprising theoretical and experimental approaches. SynBio plants can be obtained using different techniques for genetic modifications and the assessment of all these is addressed in Regulation EU No 503/2013, in EFSA guidance documents as well as in other scientific opinions (e.g EFSA GMO Panel, 2020). Moreover, Synbio does not apply to make differentiation among applications of NGTs. See also replies to comments 6, 7.

9	1.2. Background and Terms of Reference as provided by the requestor	ANSES	No comment	Comment noted.
10	1.2. Background and Terms of Reference as provided by the requestor	CropLife Europe	Line 60: Not clear what "and at international level" is referring to. Please provide reference in support.	The term is proposed in the EC mandate to EFSA (see Section 1.2 of the scientific opinion).
11	1.2. Background and Terms of Reference as provided by the requestor	BfN - Federal Agency for Nature Conservation	Lines 79 ff Post-market environmental monitoring (PMEM) is not explicitly included in the six work packages. However, PMEM is an integral part of the approval procedure. Therefore, the adequacy of existing guidelines for the monitoring of SynBio plants should be covered in this issue, as well. PMEM builds upon the results of the ERA. Its aims are to confirm the results of the ERA, to identify adverse effects that were not anticipated in the ERA and to detect cumulative, long-term effects. Due to the nature of the interplay of ERA and PMEM, it is important to analyze the adequacy of guidelines for the PMEM parallel to the adequacy of the ERA, as aspects should be prevented from shifting from ERA to PMEM. All steps of the tiered-based approval procedure need to be finished completely, before the next step can be undertaken. Currently applied monitoring methods of GMP authorized for food and feed focus on preventive measures such as HACCP. However, potential entry points into the environment such as e.g. transport routes or the surroundings of processing and transshipping facilities should be additionally subjected to a scientific monitoring of environmental effects. Zünd et al. (2019) provide a conceptual framework for such a monitoring and the VDI-guidelines provide standardized monitoring methods (http://www.vdi.eu/engineering/vdi-standards/). These conceptual considerations are also valid for the PMEM of SynBio plants and should be added to EFSA?s considerations regarding the PMEM. Zünd J., Wüst-	PMEM has been addressed in the previous opinion (EFSA GMO Panel, 2021a), and it is not in the scope of the current opinion.

			Saucy A.G., Züghart W., Bühler C. (2019). Monitoring of Spontaneous Populations of Genetically Modified Plant Species in the Environment - Experiences and Recommendations for the Design of a Monitoring Programme. Technical Report forEPA, ENCA, IGGMO.	
12	1.3. Interpretation of the Terms of Reference and scope	ANSES	No comment	Comment noted.
13	1.3. Interpretation of the Terms of Reference and scope	National Food Institute, Technical University of Denmark	General statements: None of the examples served to provide a better understanding of what might be a real new challenge. To define some of the GMP as also included in newly weak defined SynBio group is not helping to the discussion on the issue (GMP which are not considered equivalent to known food/feed plant?). It would be a misunderstanding to preclude that a plant developed by EFSA definiton of SynBio would be more complicated to evaluate or have a higher risk. A single gene could make significant changes in a plant. This should be explained in the text somewhere. The value of the opinion for risk assessment is considered quite limited and may even contribute to confusion.	Synbio is a process or strategy comprising theoretical and experimental approaches, not a technique, or a combination of techniques (see also reply to comment 8). The horizon scanning previously conducted (EFSA Scientific Committee, 2020 and EFSA GMO Panel, 2021a) highlighted that GM plants obtained through Synbio reaching the market in the next ten years are likely to result from existing technologies including those resulting to the insertion of transgenes and genome editing. However, SynBio strategies applied during the development process would enable for more complex traits. Indeed, SynBio approaches are typically not being applied to achieve simple gain-of-function traits encoded by a single gene such as herbicide tolerance or pest resistance. In contrast, SynBio approaches are being applied to engineer complex, quantitative traits controlled by multiple genes (e.g. photosynthetic capacity and nutrient use efficiency); for the design of traits that require lengthy multigene pathways (e.g. to produce new metabolites); and for the de novo design of proteins able to perform new or expanded functions. The GMO Panel has also previously concluded that increased complexity and diversity of the new traits is expected in future SynBio applications compared to traditional applications, therefore challenging current risk assessment approaches. The specific Synbio GMP case studies were selected because, even though they have been produced using existing GM technologies including those resulting to the insertion of transgenes and genome editing (and therefore resemble current GMPs) their complexity is likely to require the application of SynBio approaches, (see also EFSA GMO Panel, 2021a). Moreover, these represent current and near future SynBio

				developments with a range of complex characteristics of relevance for food and feed risk assessment. The GMO Panel agrees that modifying a few genes, significant changes in the plant can occur (see case study 4). Regarding the comment on the limited value of the opinion, it is reminded that, as per EC mandate, the WP4 Scientific Opinion is intended to evaluate existing guidelines for their adequacy for the food and feed risk assessment of genetically modified plants obtained through synthetic biology, and not to serve as a stand-alone guidance document.
14	1.3. Interpretation of the Terms of Reference and scope	CropLife Europe	Lines 97-99: We agree that the focus of the assessment should be on applications that are likely to reach the market in the next 10 years, by 2030. We note that the 4 case studies selected by EFSA are examples of research applications, and not examples of potential products. We recommend that the text is amended with a note to underline the hypothetical nature of the selected cases. Line 116: we would like to highlight that the consideration of "fully fit for purpose" may need to be rephrased taking into account that whereas there is no need for additional guidance, in some circumstances EFSA acknowledged requirements are not applicable or need to be re-considered based on not being relevant or adding limited value. We note from experience that the major challenge with the existing guidance from EFSA is not its content per se, but the lack of flexibility in how it is applied. This will be true for any future products too and we recommend that "flexibility of implementation" is also considered as part of "fit for purpose" guidance.	Comments noted. The hypothetical nature of the case studies is well-mentioned throughout the scientific opinion. Editorials implemented as needed. See also reply to comment 7.
15	1.4. Summary of the conclusions of the previous SynBio opinion (EFSA GMO Panel et al., 2021)	Federal Office of Consumer Protection and Food Safety (BVL) (National Authority)	Does "engineered organisms" mean "genetically modified organisms" in this context? If so, it is recommended to use the full term "genetically modified organisms" If not the meaning of the term "engineered organisms" needs to be specified and an explanation given why it is used.	Comment noted. See reply to comment 1.
16	1.4. Summary of the conclusions of the previous SynBio opinion	ANSES	PGM are yet tested in different environments as the culture trials are realized in different parts of a country or different countries to take into account environmental variability of climate and soils.	According to the requirements of the Regulation (EU) No 503/2013 and EFSA guidance documents on ERA and agro/pheno (2010, 2015), applicants are required to perform field trials for the agronomic/phenotypic and compositional characterisation of GMPs gathering data from field trials

	(EFSA GMO Panel et al., 2021)			performed at multiple sites under conditions representative of the receiving environments in which the GMP can be grown. The EFSA GMO Panel considered these requirements adequate also in case of SynBio GMP. As reported in section 3.2.11 of the Scientific Opinion on SynBio GMP-MC-ERA (EFSA GMO Panel, 2021a) among other consideration it was stated that in case of complex traits or in case of multiple novel traits have been conferred, the selection of the relevant endpoints to be assessed may need to be adapted on a case-by-case basis. It was also stated that more emphasis may have to be put on investigating potential interactions related to the management of generated SynBio GMPs across the environments in which they could be cultivated. This may be done by considering different genetic backgrounds across various receiving environments but this would entail challenges in the practical implementation and analysis. Therefore, a more predictive approach that models the behaviour of a given construct in a specific background and in a given receiving environment might be considered by taking advantage of the advancement of approaches in ecosystem modelling.
17	1.4. Summary of the conclusions of the previous SynBio opinion (EFSA GMO Panel et al., 2021)	National Food Institute, Technical University of Denmark	Why is some words in bold? We do not find there is a link between the quantitative differences (if any?) between "traditional GMP" and GMP that might also be called SynBio and the expected risk. The risk is more related to the expected intended changes (qualitative differences). Therefore no need to emphazise these not relevant (in this aspect for risk assessment) or documented differences.	Comment noted, text edited. See also reply to comment 13.
18	1.4. Summary of the conclusions of the previous SynBio opinion (EFSA GMO Panel et al., 2021)	CropLife Europe	Line 125: We highlight that the reference to "established techniques of genetic modification" lacks precision and is misleading as tools and techniques are constantly evolving and it is not accurate to talk about "established" techniques when referring to the toolbox used 5 or 10 years ago in comparison to today. Lines 126-130: While this is referring to content in a previous opinion, this content speculates on future technological possibilities and "future challenges" for molecular characterisation. This is not relevant or consistent with maintaining a "near-future" scope of consideration. Lines 128-132. We recommend deletion of the sentences between 128-130 lines and continuing with the text in the sentence starting on line 132. The references to "scale of the changes", "large increase in the complexity", "diversity of the new traits" is very speculative and even in the original	Comments noted. For the purpose of this mandate the term 'established techniques of genetic modification' has been operationally introduced to refer to various genetic engineering techniques that have been significantly used over the last 30 years to produce genetically modified organisms, such as those that have been authorised under Directive 2001/18/EC and Regulation (EU) No 1829/2003 (see footnote 5 in the Scientific opinion). Editorials implemented as needed.

			opinion, these notions were not clearly defined or separated from what the authors called "traditional" applications. These concepts need to be defined and cannot be based on speculative judgement of what constitutes "significant" development. EFSA should refrain from basing its opinions on such qualifiers and should stick to the factual information, taking into account broader benchmarks than first generation GM crop plants.	
19	1.4. Summary of the conclusions of the previous SynBio opinion (EFSA GMO Panel et al., 2021)	Euroseeds	Line 125: We highlight that the reference to "established techniques of genetic modification" lacks precision and is misleading as tools and techniques are constantly evolving and it is not accurate to talk about "established" techniques when referring to the toolbox used 5 or 10 years ago in comparison to today. Euroseeds notes that the case studies considered representative of "synthetic biology" GMPs in the EFSA draft opinion, closely resemble GMPs achieved using "existing transgenic and non-transgenic GMO technologies". Euroseeds therefore agrees with the conclusions of the GMO Panel that had not identified novel potential risks in terms of the impact of SynBio GMPs on humans, animals and the environment, and no novel hazards. In line with the above, we encourage EFSA to further advance the consideration of derogations from certain data requirement outlined in the Implement Regulation 503/2013 that may not be relevant even for transgenic plants. Depending on the product, certain requirements may not be needed or may need to be adapted, consistent with the fundamental principle of a case-by-case approach and fit for purpose risk assessment. We emphasise that this does not correspond to a need for additional guidance. Rather, as technology evolves, data requirements should be sufficiently flexible to accommodate what is appropriate, consistent with the fundamental principle of a case-by-case approach. Also, as rightly stated in the EFSA draft 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 compared to both SDN-3 and conventional breeding techniques, including conventional mutagenesis". This fact underlines the need for a sufficiently flexible and proportionate case-by-case approach.	Comments noted. Regarding the term "established techniques", please see response to comment 18. Regarding "flexibility" or "case-by-case" approach to the risk assessment of GMPs, it is noted that this is one of the pillars of risk assessment of GMPs in the EU and it is mentioned throughout this opinion, as well as recently in other GMO Panel outputs (EFSA GMO Panel, 2020, 2021a). See also reply to comment 14.

20	1.4. Summary of the conclusions of the previous SynBio opinion (EFSA GMO Panel et al., 2021)	BfN - Federal Agency for Nature Conservation	Line 123 - 142 We do not share the conclusions and refer to our previous comments on EFSA (2020) and EFSA (2021). We agree with Unkel (2020), who remark that many scientists would not consider this kind of work on metabolic engineering as being synthetic biology. This particularly refers to case studies 2 and 4 where complex modifications without transgene insertion were reached using multiplexed gene editing. Both cases should have been dealt with in EFSA (2020), i.e. the opinion on SDN-1, SDN-2 and ODM. In our comment on EFSA (2021) we disagreed with the view that mutations deriving from conventional breeding, mutagenesis or SDNs are principally similar, because it disregards the potential of GE, which includes to a) access the whole genome for changes and even protected sites, b) alter several gene copies or related genes in parallel and c) overcome linkage drags. Next to Kawall (2019), Kannan (2018) and Sánchez-León (2018), further relevant publications are Braatz (2017), Kawall (2021) and Monroe (2022). Therefore, it cannot be assumed a priori that transgen free GE plants could occur naturally or by conventional breeding, but they require a case by case risk assessment considering both the characteristics of the product and the process (Eckerstorfer 2021; Kawall 2021). We also pointed out that sequencing the target locus is insufficient to identify unintended genomic modifications in general. Apart from off-target effects, a surprisingly large variety of on-target effects (ON-TE) near or around the target site have been reported for human and animals cells , (e.g. Boutin 2022; Kosicki 2018; Weisheit 2020; Zhang 2019) and also for plant cells (Sánchez-León 2018, examples in Hahn 2019). Their number and occurence is likely underestimated for methodological reasons (e.g. Hahn 2019; Boutin 2022). We request that ON-TE are fully considered during risk assessment and agree that suitable tools to detect them should be rapidly developed (Boutin 2022).	These comments relate to GE in plants and have been extensively revised in EFSA opinions (EFSA GMO Panel 2020 and 2021a). The specific Synbio GMP case studies were selected because, even though they have been produced using existing GM technologies including those resulting to the insertion of transgenes and genome editing (and therefore resemble current GMPs); however, their complexity, in terms of process and product, is likely to require the application of SynBio approaches, (see also EFSA GMO Panel, 2021a).
21	2.1. Ad hoc expert Working Group and its methodology	ANSES	No comment	Comment noted.

22	2.1. Ad hoc expert Working Group and its methodology	CropLife Europe	Line 149, Table 1 comment on the methodology for expanding the pool of case studies: We agree that hypothetical cases can be useful for evaluating the application and completeness of risk assessment methodologies and guidance. We disagree that the cases represent examples of products that may reach the market in the near future. We recommend that EFSA makes a clear acknowledgement of the difficulty to identify relevant cases from this point of view. This has implications for the conclusions that EFSA is presenting later on, and puts into question the need for development of additional guidance in anticipation of such new developments.	Comment noted. See also reply to comment 14.
23	2.2. Selection of case studies to address WP4	ANSES	No comment	Comment noted.
24	2.2. Selection of case studies to address WP4	National Food Institute, Technical University of Denmark	It is quite disappointing that these examples were not self-evident and clear taken into account during the development of the EFSA guidelines many years back. All the cases should have been foreseen (and was by many) and are the result of traditional genetically engineered in plants whether or not you can by a definition add a new name (SynBio) to them. It seem as if EFSA are complicating these cases and the issue here. As stated before during a previous hearing "It is therefore confusing to define these classical cases as examples of SynBio just because they are more complex than many of the previous applications in EU according to EFSA." The statement "the technological complexity of these case studies would require the application of SynBio approaches" is just not scientific well-founded and the "SynBio approaches" is not clear what is mend. Define when something is technological complex. Genetic engineering is complex. By using examples that should have been covered by the guidelines and can be considered as conventional GMPs this will not bring new guidelines (if needed) a step forward. It may also confuse the managers to think they will represent future SynBio e.g. a GMO that could be considered as a novel food plant. For novel food there are no conventional counterpart and hundreds of new (not yet defined) proteins ? that will be the challenge and worthwhile to consider.	Comment noted. Synbio is a process or strategy comprising theoretical and experimental approaches, not a technique, or a combination of techniques and SynBio approaches are typically not being applied to achieve simple gain-of-function traits encoded by a single gene such rather to engineer complex, quantitative traits controlled by multiple genes potentially challenging current risk assessment approaches. The specific Synbio GMP case studies were selected because, even though they have been produced using existing GM technologies including those resulting to the insertion of transgenes and genome editing (and therefore resemble current GMPs); however, their complexity, in terms of process and/or product is likely to require the application of SynBio approaches, (see also EFSA GMO Panel, 2021a). Moreover, the chosen case studies were considered suited for the purpose since representing current and near future SynBio developments showing a range of complex characteristics of relevance for food and feed risk assessment. See replies to comments: 13, 17, 23, 25.

25	2.2. Selection of case studies to address WP4	Euroseeds	Lines 154-155: Uses the term "conventional techniques" in reference to transgene insertion and genome editing. This is confusing, since the general use and understanding of this term in plant breeding is different as outlined by EFSA (EFSA Journal 2012;10(10):2943 in which also van der Wiel, 2010 is referenced. We encourage EFSA to be consistent in view of the use of certain terms and definitions. Lines 155-156: States that it is not the ("conventional") technique used, but the technological complexity of the approach that qualifies a case study as being synthetic biology. There is an apparent lack of consistency in the definition of synthetic biology over the (now several) documents on the topic. This inconsistency is confusing, and it is not clear at what point a case study is sufficiently ?complex? to become synthetic biology. According to our understanding at least case study 2 and 4 do not match the SynBio criteria as outlined in the report. Moreover, since the application of targeted genome editing methods resulted in point mutations in case study 2/4 it is unclear how such a plant would be distinguishable from a conventionally bred variety with identical point mutations and with that be identifiable as a product of SynBio applications. This is also specifically mentioned for case study 4 in line 172 (material resembles the characteristics of cultivated tomato varieties). Line 175. In line with table2/case study 4 consider the wording "targeted gene editing" for case study 2 table 2/column technology instead of "non-transgenic" for the gluten-free wheat.	Comments noted, text edited. Regarding the term "conventional techniques", please see response to comment 18. Synbio is a process or strategy comprising theoretical and experimental approaches, not a technique, or a combination of techniques. The horizon scanning exercises previously conducted (EFSA Scientific Committee, 2020 and EFSA GMO Panel, 2021a) highlighted that GM plants obtained through Synbio reaching the market in the next ten years are likely to result from existing techniques including those resulting to the insertion of transgenes and genome editing. However, SynBio strategies applied during the development process would enable for more complex traits. The specific Synbio GMP case studies were selected because, even though they have been produced using existing GM technologies including those resulting to the insertion of transgenes and genome editing (and therefore resemble current GMPs); however their complexity, in terms of process and/or product, is likely to require the application of SynBio approaches, (see also EFSA GMO Panel, 2021a). Moreover, these represent current and near future SynBio developments with a range of complex characteristics of relevance for food and feed risk assessment. See also replies to comments 13, 17, 23.
26	2.2. Selection of case studies to address WP4	German Central Committte on Biological Safety (ZKBS)	See uploaded file for comments.	Comment noted.
27	2.3. Existing guidance documents and guidelines considered in this Opinion	ANSES	No comment	Comment noted.
28	2.3. Existing guidance documents and	CropLife Europe	Line 193-19: It is not clear how the relevance of the documents for the food and feed risk assessment was established.	Comment noted, text edited.

	guidelines considered in this Opinion			
29	2.4. Consultation	ANSES	No comment	Comment noted.
30	3.1. General outline to food and feed risk assessment of GMPs	ANSES	The complexity of Synbio PGM and their overall difference from plants commonly consumed might prevent finding a genetically similar counterpart with HoSU.	Comment noted.
31	3.1. General outline to food and feed risk assessment of GMPs	National Food Institute, Technical University of Denmark	It is stated (line 244 -) that the WG considered that assessment was originally set for "conventional GMPs". We do not agree. Please provide documentation for this statement was indeed the thinking years back. We consider that guidelines should covering cases that easily could be foreseen coming in the future. This is the cases for the examples provided which we have foreseen in many years. Instead of looking at the very strict EFSA comparison concept as the only way forward in the risk assessment EFSA should have been more flexible and left some room for other ways forward when needed. This was e.g. done in relation to the novel food regulation 97/258 where a simplified and flexible procedure possible based on substantially equivalence in the comparison "on the basis of the scientific evidence available and generally recognized or on the basis of an opinion delivered by one of the competent bodies referred to in Article 4 (3), are substantially equivalent to existing foods or food ingredients as regards their composition, nutritional value, metabolism, intended use and the level of undesirable substances contained therein. The conclusion should thus be "sufficient" when talking about whether the existing guidelines can be used for the risk assessment of all examples. Included in "sufficient" would also be for cases where the case-by-case "procedure" are used and questions are asked that had not previously been asked. This happens many times as well as the involvement of new informations.	SynBio approaches are typically not being applied to achieve simple gain-of-function traits encoded by a single gene such as herbicide tolerance or pest resistance. In contrast, SynBio approaches are being applied to engineer complex, quantitative traits controlled by multiple genes (e.g. photosynthetic capacity and nutrient use efficiency); for the design of traits that require lengthy multigene pathways (e.g. to produce new metabolites); and for the de novo design of proteins able to perform new or expanded functions. Furthermore, the GMO Panel has previously concluded that increased complexity and diversity of the new traits is expected in future SynBio applications compared to conventional GMPs. Already in 2011 the GMO Panel noted that increasing complexity of GMPs could challenge the identification of comparators and consequently comparative analysis, and introduced flexibility and options, calling at the same time for the need of further development of comprehensive safety and nutritional assessment of such GMPs (see EFSA GMO Panel 2011b: "The EFSA GMO Panel has, to date, required as comparators either non-GM lines with a genetic background as close as possible to the GM plant under assessment in case of sexually propagated crops, or isogenic varieties in case of vegetatively propagated crops. The identification and production of such comparators is becoming increasingly challenging due to the increasing complexity of GM plants, e.g. those developed by combining stacking) events through conventional breeding, or those in which significant compositional changes are targeted. The EFSA GMO Panel also considers situations where additional comparators may be required on a case-by-case basis and scenarios where appropriate comparators are not available (e.g. where extensive compositional changes are targeted). Whilst considering the requirements of Directive 2001/18/EC and

				Regulation (EC) No 1829/2003, the EFSA GMO Panel provides options which introduce flexibility in the selection of comparators based on sound scientific principles).
32	3.1. General outline to food and feed risk assessment of GMPs	Euroseeds	Line 244-255 "Conventional" GMPs are typically designed to bring new traits (such as insect resistance, herbicide tolerance or compositional changes)? Euroseeds would like to point out here that the use of the term "conventional" in the context of GMPs is confusing. We would rather ask EFSA to consider using the term "transgenic" in the sense of earlier EFSA reports (Transgenes are DNA fragments outside the breeders gene pool which includes sources of genes available for conventional plant breeding as outlined by EFSA in EFSA Journal 2012;10(2):2561). In addition, the reference of insect resistances, herbicide tolerances or compositional changes in view of "new traits" in the context of "conventional GMPs" is misleading. The development of all these traits is not restricted to a certain set of breeding methods and are with that not new traits developed only since the use of "conventional GMPs". Lines 254-255: the paragraph continues with stating that "synbio GMPs" may be complex and overall significantly different from plants commonly consumed". This is speculation that is not supported by the 4 case studies and specifically case study 2 (several mutations were introduced that reduce the amount of proteins that have a history of safe consumption in the EU. A pure reduction of such proteins should not justify any risk analysis) and the conclusions presented in this opinion.	Comments noted. The complexity of a Synbio GMP may or may not be associated to an analogous complexity in characteristics of relevance for food and feed, such as its composition. SynBio approaches are typically not being applied to achieve simple gain-of-function traits encoded by a single gene such as herbicide tolerance or pest resistance. In contrast, SynBio approaches are being applied to engineer complex, quantitative traits controlled by multiple genes (e.g. photosynthetic capacity and nutrient use efficiency); for the design of traits that require lengthy multigene pathways (e.g. to produce new metabolites); and for the de novo design of proteins able to perform new or expanded functions. Furthermore, the GMO Panel has previously concluded that increased complexity and diversity of the new traits is expected in future SynBio applications compared to GMP applications assessed to date. Editorials implemented as needed.
33	3.1. General outline to food and feed risk assessment of GMPs	BfN - Federal Agency for Nature Conservation	Line 222 / 223 The existing guidelines are not sufficient for a comphrehensive molecular characterisation which is one of the starting points to structure and conduct the RA of GMPs (cf. comments on 1.4). Line 228 - 229 This is irritating, as MC and comparative analysis aim to identify and characterize intended and possible unintended effects (u.e.) linked to the genetic modification. Whether they succeed is depending on the specifications which we find insufficient (see comment on line 231 ? 235). The statement should express an aim rather than a matter of fact. Line 231 - 235 The four case studies pose new challenges. i.e. (i) The	Comments noted. The adequacy of the existing guidelines for the Molecular characterisation of GMPs obtained by Synbio has been previously addressed (EFSA GMO Panel et al. 2021) and it is out of the scope of this opinion. Editorials were implemented as needed.

			number of NEPs can be quite high, e.g. up to 25 in case study 1; (ii) SynBio plants are likely more complex with several new traits and more and complex interactions with the plant metabolism leading to numerous new compounds/altered levels of constituents; (iii) Quite likely not all of them will be captured by the compositional analysis of preselected compounds; (iv) Due to the complex intervention, the number of u.e. due to the genetic modification is likely to rise. The prevailing concept of FF risk assessment is mainly based on the idea that GMPs have a limited number of traits and transgenes with risks posed by NEPs and new constituents and obvious u.e. This concept might be manageable for single events with a single transgene. However, it should be recognized that with complex SynBio plants and multiplexed NGT plants, MC and comparative assessment of selected parameters and endpoints are insufficient and existing guidelines need to be accompanied by non-selective screening methods such as omics at various organizational levels. Line 253 The term "conventional" GMPs is misleading, as they do not derive from conventional breeding and have no HOSU. Instead they could be called present GMPS or current GMPs.	
34	3.1. General outline to food and feed risk assessment of GMPs	Testbiotech	The draft from EFSA misses to address potential unintended changes in agronomic or phenotypic characteristics, even though this is part of existing guidelines for risk assessment of food and feed safety and Implementing Regulation 503/2013. This should, therefore, be fully addressed in the context of SynBio plants, the case studies have to be revised accordingly.	Possible unintended changes in agronomic and phenotypic characteristics on SynBio GMP are discussed in the Scientific Opinion (EFSA GMO Panel, 2021a). The discussion covered the identified case studies as well as hypothetical more complex situations (see section 3.2.11). Those considerations remain valid also for the additional case study discussed in the current opinion.
35	3.2.1. Case study 1 Vitamin B12-producing maize	ANSES	The interest of integrating these new genes and traits into the plant should be argued and justified by the petitioner.	Comment noted. The GMO regulatory frame requires that for any GMPs applicants provide a general description of the introduced trait(s) or modified information and its mode of action, of the resulting changes on the phenotype and the metabolism of the plant, and of its intended use. See also replies to comments 61, 85
36	3.2.1. Case study 1 Vitamin B12-producing maize	CropLife Europe	Lines 266-271: The use of hypothetical examples is good way to challenge the existing guidelines. However, as it is a hypothetical example, it runs into speculation pitfalls. As the GMO panel report states, these are examples that have the potential (COULD) to reach market within the next ten years, so it is not an assurance. First, what is considered as a	The GMO Panel selected these specific Synbio GMP case studies (including case study 1) based on a tailored horizon scanning of recent literature and because hypothesised that their complexity may require engineering principles such as standardization, modularity, modelling and computer-aided design to improve the predictability of the bioengineering process.

			SynBio example at the moment may not be in the future. Furthermore, as stated in the GMO panel scientific opinion, the ability to introduce B12 pathway in plant is likely to relay on what EFSA describes as "synbio" approaches including in silico modelling and prediction of pathways and which seems to be the basis for the perceived complexity of the example. The use of computational tools, however should not automatically convert the example into a SynBIo product. Lastly, there is a product on the market OMEGA-3 Canola, which introduced a number of genes from algae into a plant assembling a pathway for the production of high levels of DHA. This product is based on publication dating to 2005 (https://pubmed.ncbi.nlm.nih.gov/32689148/) and this was not considered a SynBio example, but the parallels with the current B12 example are clear. Perhaps this could have been a better example to test the guidelines as there is ample experience with the introduction of bacterial genes into crop plants (Bt corn, Bt maize).	Indeed, the application of modelling and computer-aided design is key and informs and predicts the outcomes of different engineering strategies; prototype testing provides experimental data that subsequently can improve the design in "design-build-test-learn" cycles. While previous cases such as the omega 3 case are relatively simple examples of genetic engineering as the enzymes introduced, intermediates and products are not completely new to plants; the Vit B12 example is of higher complexity, requiring synthetic biology approaches. It deals with a completely new molecule, not existing in the plant kingdom, requiring alien cofactors, and of high molecular/structural complexity. A high number of enzymes are required (no example yet of such a large and complex pathway successfully being introduced into plants), there are no analogue enzymes on the plants and the substrates and intermediates are also "alien". Modelling will in addition be required to instruct the design, in terms of promoters needed to achieve the optimal expression level for each enzyme, sub cellular compartmentalisation, and other relevant aspects.
37	3.2.1.1. Comparative analysis	ANSES	The comparator should be specified. Logically, according the guidelines it should be the conventional counterpart as close as possible of the evaluated PGM. The evaluation of compositional analysis should be completed with the data of comparison for agronomic and phenotypic characteristics giving also information on possible modification of biological characteristics of maize.	Comment noted.
38	3.2.1.1. Comparative analysis	National Food Institute, Technical University of Denmark	Line 285: "Information from the SynBio product design and optimisation (e.g. "omics data") could support the.." Either it should be deleted or explaned in more details. What is meant by "Information from the SynBio product design"? It would be identical to ask for the function of inserted genes and their involvement in synthetic pathways etc. and ask for information that may document their function? Concerning the mentioned "omics data" this is not the first time some would like to "sell" the omics-technique. First of all there are different "omic-" techniques. None of them have been found to useful (validated) for risk assessment but useful for other purposes. It should be avoided to give the (false) impression	The knowledge available from the SynBio design and modelling could effectively anticipate the expected characteristics of the SynBio product and inform risk assessors of the selection of further analytes that might be relevant for food and feed safety. For example, data obtained during the experimental validation of a Synbio prototype can be useful for risk assessors. The GMO Panel indicates that the use of such information for the risk assessment should be integrated into future guidance documents. Omics is among the tools that can be used and it is proposed as example.

			that these techniques may play a role in future risk assessment. This has happened before.	
39	3.2.1.1. Comparative analysis	CropLife Europe	Line 273: Correct typo (delete "on"). Line 276: Suggestion to delete the expression "SynBio maize" and use GMO maize instead, in alignment with the information summarized in table 2. We caution against the use of the "SynBio" qualifier as we disagree with the interpretation that this is a representative synthetic biology application, and are concerned of EFSA perpetuating concepts that are not fully justified (as was acknowledged in the original case studies). Line 281-282: Suggestion to delete "The analytical techniques to be used will depend on the type and number of analyte/s to be measured". As long as the requirements of the existing EFSA guidance documents and EU legislation are followed, the analytical technique used is up to the discretion of the applicant. Lines 285-287: This implies the need for future guidance documents in addition to the current guidance documents. A full description of the product and methods is already included in current guidance. If additional guidance document is required, reviewing and bringing up to date existing but outdated requirements should be considered as a priority.	Comments noted, text edited as necessary. Reagrding the comment on analytical techniques, the text mentioned is in line with Reg. (EU) No 503/2013: "The specific analyses required shall depend on the plant species examined but shall include a detailed assessment appropriate to the intended effect of the genetic modification, the considered nutritional value and use of the plant" (Annex II, II, 1.3.4. Comparative analysis of composition).
40	3.2.1.1. Comparative analysis	BfN - Federal Agency for Nature Conservation	Line 277 / 278 We do not agree that the addition of just "vitamin B12" to the maize compositional endpoint list (OECD, 2002) would be sufficient for an appropriate compositional analysis, but all the substrates and metabolites of the inserted pathway need to be analyzed as well. 25 genes were required to synthesize vitamin B12 in non-B12 bacteria, so several interactions with the endogenous metabolism of the vitamin B12 maize can be assumed and likely not all of them will be captured via selected endpoint analysis. We do not agree that the existing guidelines are sufficient and recommend to include omics methods as a non-selective screening step (see comments on lines 231 / 235). Line 283 284 The vitamin B12 maize has a high number of transgenes and expresses several NEPs for an entirely new pathway. It is particularly challenging to predict all potential links to the endogenous plant metabolism and the impacts when the GMO is grown in open field trials. Therefore, field trial data should in any case be analyzed for any interaction between the GM material and	Comments noted. In the context of this scientific opinion, "omics" is mentioned as one among the possible techniques used in the Synbio development (design and development). The GMO Panel considers that information from the SynBio product design and optimisation could effectively anticipate the expected characteristics of the SynBio product and inform risk assessors of the selection of further analytes that might be relevant for food and feed safety. Appropriate analytical techniques will then be applied, as needed in line with Reg. (EU) No 503/2013 (Annex II, II, 1.3.4. Comparative analysis of composition and comment 39). The GMO Panel also recommends to integrate into future guidance documents the use of such information for the risk assessment.

			the site, i.e. the receiving environment. The existing guidelines should be strengthened and clarified in this respect. If the growing conditions have a significant impact on the composition of the vitamin B12 maize, it should be considered to use GM material from different sites for the toxicity study.	
41	3.2.1.1. Comparative analysis	Testbiotech	Example 1 shows that the complexity of the newly introduced metabolism and the high number of additional proteins expressed in the plants results in a new quality of hazards and risks. The complexity of the introduced changes as well as the unintended changes in the plant genome and metabolism are not unlikely to cause specific patterns of unintended changes in the genetic and metabolic networks. Furthermore, these changes may be influenced by environmental factors that are known to impact gene expression. There is thus a need for comprehensive methodology to assess changes in plant composition and phenotypic characteristics, which also makes use of "omics" (genomics, transcriptomics, proteomics, metabolomics). In addition, the plants should be exposed to a sufficiently broad range of biotic and abiotic stressors to investigate the extent to which these factors impact plant composition, phenotypic characteristics and gene expression. Existing guidance neither provides nor requests the necessary methodology. Therefore, the current guidance cannot be regarded as adequate or sufficient.	Comment noted. See reply to comment 38.
42	3.2.1.2. Toxicology	ANSES	If the HoSU of the genes-sources and of the newly expressed proteins is not demonstratable, a 28-day study is suggested by the WG. However, testing in vivo each new protein is not relevant in tne case of numerous proteins, because of the 3Rs, and because assessment of the proteins interaction will be also needed. Assessing each protein individually could be waived if the combination is assessed in a 28-d study, or at least in vitro (à discuter). Numerous proteins newly expressed will have to be evaluated. The GMA panel propose to conduct combination studies if necessary. Instead testing the possible toxic effect of each protein may be testing directly the combination of all new proteins will be more relevant. The GMA panel considers that new alternative methods would have to be developed and validated for assessing numerous new proteins. These developments	Comment noted. It is reminded that this opinion is not intended to serve as a new guidance document, so no technical details how to conduct the toxicological assessment of newly expressed proteins is provided.

			need time and the Synbio PGM are yet present. A 28-day toxicity study with the combination of all new proteins could be required. The 90-day toxicity study in rodents allows testing the possible interactions between new proteins and the plant's original metabolic pathways within the matrix that will be consumed. The whole feed study on animals is also complementary. The 90-day study is of added value compared to the comparative analysis, but shows a limited sensitivity.	
43	3.2.1.2. Toxicology	National Food Institute, Technical University of Denmark	EFSA claims that the strategy for the toxicological assessment was developed for GM crops expressing a limited numbers of new proteins. This is news for us and we would like to know how many proteins were included? It does not make sense since for stacked events there are no limitations to our knowledge of how many (DNA) constructions could be involved in the stacked plant and EFSA require all the single events to be assessed before the stacked event will be worked on. This include the assessment of all the new proteins including animals test of those without history of safe use. We have before argued against this non-scientific assessment of all stacked event. Would EFSA consider naming stacked events with many new proteins to be SynBio? Do EFSA by the text in this chapter indicate that the demand of data should be less when there are a higher number of new proteins? Would EFSA accept less data for risk assessment due to the work load. We do not understand the (scientific?) principles of EFSA. Maybe the text should be more clear on this. It could be red as if there are no new challenge here except for more work and data to be provided. How should this influence on risk assessment?	In case study 1, about fifteen genes are introduced to synthetise corresponding enzymes producing the target metabolite (vitamin B12). In future applications aiming to produce more complex metabolites or several different metabolites, much more numerous genes (and corresponding proteins) could be introduced. Although, in principle the same techniques used for assessing one protein can be applied, in practice it will be challenging to perform toxicological studies on numerous newly expressed proteins. See further details in section 3.2.1.2 of the scientific opinion. See also reply to comment 77.
44	3.2.1.2. Toxicology	BfN - Federal Agency for Nature Conservation	Line 357 - 360 The Panel's opinion that a 90-day study on the whole food and feed of vitamin B12 maize would not provide any added value for the safety assessment on the ground that there are no toxicological concerns associated with excess vitamin B12 is incomprehensible against the background that problems and challenges with the toxicological assessment of a high number of NEPs have already been identified in this chapter. Line 360 - 363 We do not agree with this statement. According to Regulation (EC) 503/2013 a 90-day feeding study in rodents with whole food or feed is the primary	Comment noted.

			additional study to address uncertainties identified in the course of the safety assessment. As the comparative analysis as well as the MC are based on preselected parameters/endpoints, non-selective methods to address unintended effects of the genetic modifications are missing and the conduction of a 90-day feeding study is justified.	
45	3.2.1.2. Toxicology	Testbiotech	The higher number of additional proteins expressed in the plants and other potentially unintended effects pose new challenges in the assessment of the proteins, both in isolation and in combination, also taking into account the emergence of other additional biological active molecules (such as ncRNAs) as well as interactions with the plants constituents and mixed toxicity. Furthermore, for example, impact on the immune system which can be effected via the intestinal microbiome has to be considered. However, existing guidelines for EFSA risk assessment, do not provide the methodology to comprehensively assess effects on the immune system, including chronic inflammation. Therefore, current risk assessment cannot be regarded as adequate or sufficient. EFSA's considerations about the necessity of 90 days feeding studies is irritating and seems to be misplaced. In general, there are many gaps in the methodology of existing risk assessment such as the reaction of the plants to biotic and abiotic stressors, the emergence of additional biological active molecules (not only proteins), interactions of the newly produced molecules (also in regard to the plants constituents), mixed toxicity, impact on the immune system, on the reproductive system and on the microbiome. Therefore, in general, more data and higher standards in risk assessment would be needed. Therefore, for the time being, the 90 day feeding studies have to be performed independently from the outcome of the comparative assessment and the molecular characterisation.	Comments noted. In relation to the microbiome and the immune system, the EFSA GMO Panel has indicated the need to invest future resources on the topic, including research activities (EFSA GMO Panel, 2022). Moreover, EFSA is engaged in horizontal development activities on the microbiome and its relevance in food and feed risk assessment (see reply to comment 73).
"	3.2.1.3. Allergenicity	ANSES	"sequence identity" seems more appropriate than "sequence homology" because the sequence analyses dealing with global (> 35% identity over a sliding window of 80 amino acids) and local (100% identity over a sliding window of 8 amino acids) searches, deal with identities and not with homologies/similarities. Resistance to pepsin and trypsin in simulated gastric and intestintinal in vitro tests; instead of	Amino acid sequence homology is the terminology used in international (Codex Alimentarius 2009) and EFSA guidelines (EFSA GMO Panel, 2011a), and Regulation (EU) 503/2013. Please also note that pepsin resistance and in vitro digestibility tests is also terminology used in Regulation (EU) 503/2013. This terminology has been used to provide a clear linked between such documents and the scientific opinion on Synbio.

			"pepsin resistance and in vitro digestibility tests" Resistance to heat denaturation add to Specific serum screening (if necessary) So far, the evaluation of the potential adjuvancity of newly expressed proteins is essentially based on a literature review together with the identities of proteins with well known toxins (which is often not conclusive because most of so-called "toxins" occurring in the NCBI nr database used for this purpose, are no adjuvant properties! Thev assessment of the potential adjuvant character of proteins expressed in GMP is left to the discretion of the evaluators. Some guidelines should be usefull to help us in evaluating more accurately the potential adjuvancity of the expressed proteins. Along this line, the evaluation of Cry 1Ac protein, for which an adjuvant character has be claimed in a few publications, is still puzzling because of very confusing results coming from different research groups dealing with the adjuvancity of Cry proteins Not convinced that the efforts being undertaken to improve the "physiological conditions" of the in vitro digestibility tests will be successful because of the extreme difficulty to reproduce in vitro both the diversity and the variability of the human digestion process. Some bioinformatic approaches dealing with the identification of peptides resulting from the complete cleavage of the expressed proteins by pepsin, trypsin and chymotrypsin (prediction with e.g. PeptideCutter - Expasy) should be so informative to predict the ability of proteins to become more/less digested in physiological conditions.	Adjuvanticity has been an area of controversy and discussion in the assessment of GMOs. The comments made in such respect would also apply to conventional GMOs and is not specific for Synbio products. Human digestion is a complex process. There have been several attempts to replicate the process in vitro acknowledging the limitations of the current models. Current and future research activities will help in providing a better understanding of the intrinsics of the process and how it can inform the risk assessment. These will also include in silico analyses for the prediction of protein digestion.
47	3.2.1.3. Allergenicity	National Food Institute, Technical University of Denmark	How the number of new individuals proteins is related to the complexity and new challenge is from a scientific point of view not clear. The function of the genes would be of importance not the number. Is it related to work load (management)? Please elaborate on this and explain the connection between number and risk. A high number of new genes/proteins is just what have been expected will be the case for some GMP.	The assessment of proteins individually might not be manageable if a large number of proteins are to be assessed. Codex Alimentarius was mainly targeted for the assessment of few newly expressed proteins. More complex future products will challenge the overall practical implementation of such guidelines, mainly targeted to assess few newly expressed proteins. More challenging applications are expected in the future with large numbers of diverse proteins, for instance, derived from new genome techniques and synthetic biology. Therefore, it is timely to review and clarify the main purpose of the allergenicity risk assessment overall and the vital role it plays in protecting consumers' health with existing food allergies and assessing the potential for foods to cause new food allergies (EFSA GMO Panel, 2022).

48	3.2.1.3. Allergenicity	CropLife Europe	Lines 381-384: CropLife Europe agrees that assessing the allergenicity of all proteins individually is not practical. Using problem formulation and the case-by-case approach, which would take account of the design and build of products (as is done in other areas), would provide adequate tools to provide information on potential allergenicity and focus the assessment on those elements that could lead to harm (hypothesis-driven). Line 382: Suggestion to delete "conventional" - we already commented that "conventional GMP" is not appropriate term. We believe that it is sufficient to refer to GMOs expressing limited number of proteins, without the need to qualify such GMOs as "conventional". Line 383-384: The use of the term "SynBio plants" is not needed and is unfortunate. We recommend that this is replaced simply with "GMPs" in the sentence.	Comments noted. Problem formulation and case-by-case approach are important concepts stressed by EFSA (EFSA GMO Panel 2017, 2021a). In relation to allergenicity, the clinical relevance of individual food allergens should be a key driver for developing news strategies and tools for allergenicity risk assessment (EFSA GMO Panel, 2022). Editorials have been implemented.
49	3.2.1.3. Allergenicity	Testbiotech	EFSA assessment of allergenic risks in existing guidance is not based on sufficiently realistic exposure to newly introduced proteins and their interactions. Different routes of exposure, the timing of exposure, microbial exposure, oral and gut microbiota composition, epithelial barrier integrity and/or non-allergenic components of the food matrix, such as immune-modulating components (adjuvants) of allergenic sources that facilitate immune responses, have to be considered. In particular, the high number of proteins additionally expressed in the plants make it essential for appropriate data to be made available. However, the necessary methodology is not provided in existing risk assessment. Therefore, current guidance cannot be regarded as either adequate or sufficient.	Current allergenicity assessment is based on internationally agreed principles (Codex Alimentarius 2009). These principles have been embedded into EFSA GMO Panel guidance documents (2011a, 2017). In such respect, EFSA is also investing efforts in developmental projects and engaging with the scientific community on allergenicity assessment to move forward the field. EFSA has been proactive in this respect and has already invested resources to advance the allergenicity prediction further. A series of EFSA procurements have been undertaken, representing significant steps forward (Mills et al., 2013a,b; Mackie et al., 2019; Parenti et al., 2019; EFSA GMO Panel, 2017, 2021a). Likewise, EU-funded research programmes, such as the ImpARAS Cost Action, EuroPrevall, iFAAM and AllerScreening projects, among others, also provide insights for improvement of existing and suggested assessment tools in the field of allergenicity assessment of foods. However, significant knowledge gaps remain, and the development of novel approaches to deal with allergenicity assessment needs to be pursued further (EFSA, 2021; EFSA GMO Panel, 2022)
50	3.2.1.4. Nutritional assessment	ANSES	No comment	Comment noted.
51	3.2.1.4. Nutritional assessment	National Food Institute, Technical University of Denmark	The whole text mirror that the example is just a "normal" GMP and adding the SynBio word to it make no difference. One sentence stating the assessment is no different from other GMO's could replace the whole chapter as it gives no	Comments noted.

			new information. This could also be stated for other chapters.	
52	3.2.1.4. Nutritional assessment	CropLife Europe	Lines 416: Suggestion to delete "SynBio" and replace with GM. Note that the lines 411-420 correctly make reference to "GM maize" or "this GM maize" and this use of terms should be aligned throughout the full text related to case study 1. Line 430: Suggestion to replace "SynBio" with "this GM".	Comments noted.
53	3.2.1.4. Nutritional assessment	Testbiotech	In the case of plants which are meant to produce new biologically active compounds (such as vitamins), and thus have beneficial effects on health, the intended as well as unintended effects and their potential implications for overall food safety have to be taken into account. However, as also existing experience with the opinions of EFSA shows, there is no appropriate methodology in place to assess specific intended health effects. Therefore, existing guidance for risk assessment cannot be regarded as adequate or sufficient.	GMO risk assessment always considers both intended and unintended effects resulting from the genetic modification and whether the identified compounds raise safety concerns or not. In the nutritional assessment the most recent available information on the different compounds, together with the outcome of the comparative assessment and replacement scenarios (if needed), will be used to conclude. However, the assessment of a particular health effect linked to a particular compound is not in the GMO remit. As an example, if there is a GM maize with high levels of Vit B12, the nutritional assessment will make use of the current knowledge of that compound (e.g. the existence of upper tolerable intake levels as defined and assessed by NDA Panel). We will not enter to discuss/assess whether consumption of Vit B12 represents, at least for certain populations, an advantage or not.
54	3.2.1.5. Dietary exposure	ANSES	No comment	Comment noted.
55	3.2.1.5. Dietary exposure	CropLife Europe	Lines 435-441: CropLife Europe considers that dietary exposure assessments should be hypothesis-driven and only conducted when potential hazards are identified. If the safety assessment of the newly expressed proteins has not identified any hazard, conducting a detailed dietary exposure assessment would not provide any added value to the risk assessment.	Comment noted. Reg (EU) No. 503/2013 (Annex II, II) identifies the exposure assessment (anticipated intake) of newly expressed proteins, other new constituents and endogenous food and feed constituents altered in levels as a result of the genetic modification, as an essential element of the genetically modified food and feed.
56	3.2.1.6. Conclusions case study 1	ANSES	In fact, the real challenge in this case study is the large number of new proteins, rising practical problems. These problems will need new alternative approaches, but not yet in routine use (in silico and in vivo, mixtures testing). The development of alternative strategies suggested by GMO panel is urgently needed by the timing, because of the Synbio rapid developments. Could the panel specify what means « aspects streamlining the risk assessment should already be incorporated into the design, modelling and	Comment noted. As described in section 4.3 of the scientific opinion (Recommendations) future guidance will need to encourage applicants to select plants to be modified, genetic parts and genetic modification processes based not only on their practicability and interoperability, but also take into account safety aspects, preferably a documented record of safe use and consumption. As a way to reduce the amount of data and studies required for the risk assessment of SynBio plants and their products, applicants should consider food and feed safety aspects throughout the SynBio design.

			validation phases of SynBio products for food and feed uses.?	
57	3.2.1.6. Conclusions case study 1	National Food Institute, Technical University of Denmark	Why did EFSA not foresee that in some cases we would see a high number of new proteins? Or why did EFSA not include this in their guidelines? Could you elaborate more on this? where is it stated that the guidelines were restricted and not covering cases that were foreseen?	Comment noted. See reply to comment 43.
58	3.2.1.6. Conclusions case study 1	CropLife Europe	Lines 445-446: Given the potential diversity of future GM products, CropLife Europe considers that it would be more appropriate for EFSA to ensure the use of problem formulation, case-by-case and weight-of-evidence approaches. Data requirements should be sufficiently flexible to accommodate what is appropriate, consistent with the fundamental principle of a case-by-case approach and fit for purpose risk assessment based on problem formulation. As such, we recommend: - Performing case-by-case risk assessments, using problem formulation to determine the relevant data requirements for each regulated GMP, including future GMPs that are categorized as developed though synthetic biology approaches . - Requests for data should be related to a credible hypothesis for a pathway to harm.	Comment noted. Currently, the data requirements for the risk assessment of GM food and feed are laid down in Regulation 503/2013. For certain products, for example those obtained by cisgenesis, the GMO Panel already indicated that more flexibility would be needed in future (EFSA GMO Panel, 2012). This position is confirmed in an updated draft opinion on the risk assessment of cisgenesis/intragenesis plants that is now under public consultation. In parallel, a new Scientific Committee guidance is under development that will include a formalized problem formulation process.
59	3.2.1.6. Conclusions case study 1	BfN - Federal Agency for Nature Conservation	We do not agree with the conclusions. The existing guidance would require further developments as regards molecular characterisation, compositional analysis and toxicology, cf. comments on chapters 1.4, 3.1, 3.2.1.1 and 3.2.1.2.	Comment noted.
60	3.2.1.6. Conclusions case study 1	Testbiotech	Existing EFSA guidance and Implementing Regulation 503/2013 are neither adequate nor sufficient to assess the risks of plants described in Example 1.	Comment noted.
61	3.2.2. Case study 2 Gluten-free wheat	ANSES	Is it really a gluten free wheat or a wheat with a low gluten content ? The interest of integrating these new genes and traits into the plant should be argued and justified by the petitioner.	Comments noted. The case study 2 is hypothetical and the Panel hypothesized that to achieve a gluten-free wheat it is likely to require SynBio approaches. The paper where this hypothetical case study is based on, is describing the development of a low-gluten wheat, the text was amended accordingly. In line with the comment, it is highlighted that the GMO regulatory frame requires that for any GMPs applicants provide a general description of the introduced trait(s) or modified information and its mode of action, of the resulting changes on the phenotype and the metabolism of the plant, and of its intended use. See reply to comment 35.

62	3.2.2. Case study 2 Gluten-free wheat	Euroseeds	Line 452: Suggestion to delete "disruptive" to align with the GMO Scientific Opinion document where the examples were chosen doi: 10.2903/j.efsa.2021.6301 and where it is described as "non-transgenic, gluten free wheat" as an example of trait with "low to medium" technological complexity. In the original document a reference to synthetic biology is made in relation to likely use "SynBio approaches to correctly identify all gliadins and glutenins in the hexaploid genome of bread wheat and to identify an engineering strategy that introduced mutations of the correct nature and positions", and this is a reference not to the actual case study, but to what EFSA anticipates to be needed to achieve the same outcome without the need for transgenesis (["....we anticipate a targeted approach to remove gluten achieved without the introduction of a transgene and all target genes to be successfully and precisely edited resulting in a gluten-free wheat"])For the current opinion, EFSA has used the case study as an example of "complexity" due to the number of introduced mutations that is "far beyond any plant previously assessed" by EFSA. As stated earlier, we do not consider that this case represents an example of synthetic biology nor that "what was previously assessed" can be used as a benchmark of "complexity". We recommend that the GMO panel consider the degree of variation within different alleles of the same gene as well as the variation that results from conventional breeding as a useful benchmark for their assessment. Line 455: Delete "SynBio", replace with gluten free wheat. Line 456: Suggestion to delete "the traditional". Please note that throughout the text "conventional" and "traditional" are used to refer to GMOs that were previously assessed by EFSA. We disagree that the fact that a GMO has been assessed by EFSA, or that it is on the EU market justifies the use of "conventional" or "traditional" as a way to differentiate with cases which have not yet been	Comments noted. See response to comment 61.
63	3.2.2. Case study 2 Gluten-free wheat	BfN - Federal Agency for Nature Conservation	Line 456 It is misleading to call GMPs developed by NGTs traditional, because so far only few of them are available on the global market and not a single one on the EU market.	Comment noted.
64	3.2.2.1. Comparative analysis	ANSES	The comparative analysis is of paramount importance and should be performed by the mass spec LC-MS/MS technique for assessing the presence of tiny amounts of gluten	Comment noted.

			proteins, because the occurrence of trace amounts in wheat products are sufficient to exert serious damages on the intestinal epithelium of celiac diseased people. The comparator should be precised. Logically, according the guidelines it should be the conventional counterpart as close as possible of the evaluated PGM. The evaluation of compositional analysis should be completed with the data of comparison for agronomic and phenotypic characteristics giving also information on possible modification of biological characteristics of wheat.	
65	3.2.2.1. Comparative analysis	CropLife Europe	Line 470: Suggestion to delete "SynBio plant" and replace with "gluten-free wheat" in line with the information provided in Table 2 and under section 3.2.2.4.	Comment noted.
66	3.2.2.1. Comparative analysis	Euroseeds	Line 470: delete "SynBio plant" and replace with "gluten-free wheat" in line with the information provided in Table 2 and under section 3.2.2.4	Comment noted.
67	3.2.2.1. Comparative analysis	Testbiotech	Example 2 shows that the complexity of the newly introduced CRISPR/Cas-induced changes results in a new quality of hazards and risks (see also Kawall, 2021). However, neither the molecular characterisation in the previous EFSA report (EFSA, 2021) nor the text provided for consultation (EFSA, 2022) reflect recent scientific findings. Therefore, we attach a short update on molecular characterisation of SynBio plants with specific relevance for this case, also including all references (see Annex). Example 2 is especially relevant when it comes to the discussion on unintended on-target effects such as also discussed in the Annex. In the case of this wheat, 35 out of 45 targeted alpha-gliadin genes were altered by CRISPR/Cas (SDN-1) to reduce gluten in food products. This may appear to be a successful and precise application of the gene scissors, however, the changes lack sufficient predictability: there are many different types of insertions and/ or deletions which are specific to each of the targeted genes. In some cases, additional DNA was inserted into the target site. This case shows that, even where changes are "successfully" introduced in the target genes, complex questions in regard to the safety of the plants need to be considered (see also Kawall, 2021): each and every targeted genetic site needs	Comment noted. EFSA previously concluded that there are no new hazards specifically linked with genome editing techniques, compared to conventional breeding and other established genomic techniques (EFSA GMO Panel 2020). As already described in the 2020 opinion, all genetic modification techniques, including in conventional breeding, can lead to unintended modifications that might cause unintended effects. EFSA stated that genome editing can induce off-target mutations, but these are fewer than those occurring with most mutagenesis techniques. Where they do occur, these changes are of the same types as those derived by conventional breeding techniques, including random mutagenesis techniques. Therefore, there is no hazard associated to off-target effects that is specific for genome editing techniques. In addition, in recent years, considerable effort has been directed to the improvement of the efficiency and specificity of genome editing technologies. Finally, backcrossing can be used to remove off-target mutations from the final product, except for those that are genetically linked to the intentionally modified locus.

			to undergo a detailed examination to investigate whether the alpha-gliadin proteins are still being produced, or if new proteins are produced unintentionally, or if any other unintended effects may occur. It should be fully taken into account that such unintended variations of genetic changes caused by New GE (new genetic engineering techniques) can be associated with novel quality in hazards and risks. By only considering the intended characteristics described in Example 2, and also assuming that existing guidance is adequate for the assessment of complex changes associated with these hazards and risks, EFSA (2022) is coming to the wrong conclusions.	
68	3.2.2.2. Toxicology	Federal Office of Consumer Protection and Food Safety (BVL) (National Authority)	The sentence is unclear in the part "except for the newly expressed proteins, when the guidelines are not applicable" as no proteins are newly expressed in this case study. It is suggested to rephrase to "except for the provisions on newly expressed proteins, when the guidelines are not applicable"	Comment noted.
69	3.2.2.2. Toxicology	ANSES	The comparative analysis will highlight the expression or production of new compounds or altered levels of constituents, needing toxicological assessment to assess potential unintended effects , in a 90-day toxicity study (to specify).	Comment noted. As described in section 3.2.2.2, if the only changes in composition of this SynBio crop are reduced alpha-gliadin levels (intended trait) and/or in compensatory effects on other glutenins (unintended effects), these would not translate into an a priori hypothesis to be tested in a 90-day study in rodents. In fact, the rat model is not sensitive to capture glutenin/gliadin-related effects of relevance for target populations. The outcome of comparative analysis would allow the identification of possible unintended effects (other than glutenins) as possible toxicological hypotheses to explore in a targeted 90-day study.
70	3.2.2.2. Toxicology	CropLife Europe	Line 473: Suggestion to delete "SynBio plant " and replace with "gluten-free wheat" in line with the information provided in Table 2 and under section 3.2.2.4.	Comment noted.
71	3.2.2.2. Toxicology	Euroseeds	Euroseeds agrees with EFSA on the conclusion that when there is no expression of new proteins no toxicological or allergenicity assessment is needed.	Comment noted.
72	3.2.2.2. Toxicology	BfN - Federal Agency for Nature Conservation	Line 473 - 474 The sentence is somewhat irritating for two reasons: (i) If no new proteins are expressed in this SynBio plant, the toxicological assessment of new proteins does not apply rather than is not needed; (ii) It should be clarified and mentioned that this only applies to new proteins which are intentionally expressed, but not to new proteins due to e.g. frame shift mutations. Line 478 - 480 It cannot be	Comments noted, text edited as appropriate.

			assumed in advance that potential unintended effects would be identified - as a matter of fact - in the comparative analysis (cf. comments on line 228 - 229). This is because the comparative compositional analysis is not comprehensive. It very much depends on the selected endpoints and the conditions under which the GM material was grown whether potential unintended effects are identified. In this context we refer to our comments to chapter 1.4 that the existing guidance is insufficient for a comprehensive MC of SynBio and NGT plants. Therefore, a) rephrasing should be considered and b) a toxicological study with whole GM material should be conducted to account for remaining uncertainties. Line 486 - 488 We do not agree with this statement. According to Regulation (EC) 503/2013 a 90-day feeding study in rodents with whole food or feed is the primary additional study to address uncertainties identified in the course of the safety assessment. As the comparative analysis as well as the MC are based on preselected parameters/endpoints, non-selective methods to address unintended effects of the genetic modifications are missing and the conduction of a 90-day feeding study is justified.	
73	3.2.2.2. Toxicology	Testbiotech	In regard to toxicology, not only the intended but also unintended effects have to be considered (see above). The draft EFSA text is misleading in this respect when it states that no new proteins are expressed in these plants and that, therefore, no toxicological assessment of new proteins is needed. The wording must be corrected to clarify that, while no new proteins are expressed intentionally in the plants, a toxicological assessment of potentially unintentionally produced new proteins (peptides) is needed. As argued above, each and every targeted genetic site needs to undergo detailed examination to investigate whether the alpha-gliadin proteins are still being produced, or if new proteins are being unintentionally produced, or if any other unintended effects may occur. Risk assessment also should take into account the emergence of other additional biological active molecules (such as ncRNAs) as well as interactions with the plants constituents. It should be fully	Comment noted, see also reply to comment 72. The considerations proposed are not specific to Synbio GMPs, rather applicable to any GMPs. The current guidelines for the risk assessment of GMP for food and feed require to identify and characterise intended/unintended effects and to assess these as regards their safety and nutritional characteristics for humans and animals (Commission Implementing Regulation 503/2013[1]). EFSA is actively engaged in evolving risk assessment methodologies based on the most recent scientific advancements (see EFSA GMO Panel, 2022 on the needs on allergenicity developments; and the EFSA horizontal thematic grant GP/EFSA/ENCO/2020/02 – MICROBIOME).

[1] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013R0503&from=EN

			taken into account that the intended as well as the unintended variations of genetic change caused by New GE are associated with novel hazards and risks regarding toxicology, even if no off-target genetic changes are identified. Furthermore, for example, impact on the immune system which can be effected via the intestinal microbiome has to be considered. However, existing guidelines for EFSA risk assessment, do not provide the methodology to comprehensively assess effects on the immune system, including chronic inflammation. Therefore, current risk assessment cannot be regarded as adequate or sufficient.	
74	3.2.2.3. Allergenicity	Federal Office of Consumer Protection and Food Safety (BVL) (National Authority)	The sentence is unclear in the part "except for the newly expressed proteins, when the guidelines are not applicable" as no proteins are newly expressed in this case study. It is suggested to rephrase to "except for the provisions on newly expressed proteins, when the guidelines are not applicable	Comment noted, text edited. See also reply to comment 72.
75	3.2.2.3. Allergenicity	ANSES	Two different approaches should be used in this respect: 1) An experimental LC-MS/MS approach to detect trace amounts of potentially deleterious immunotoxic peptides and, 2) In silico docking experiments of the identified putative immunotoxic peptides derived from residual gliadins and other LMW glutelins, to get an insight into their ability to properly interact with the basket of HLA-DQ2 and HLA-DQ8.	Comment noted. It is reminded that this opinion is not intended to serve as a new guidance document, so no technical details how to conduct the risk assessment of newly expressed proteins is provided.
76	3.2.2.3. Allergenicity	National Food Institute, Technical University of Denmark	We don't find the number of genes expressing proteins would "challenge the approach currently followed". We talk about quantity and not differences in quality. We have already seen cases with several proteins with now positive opinion. How many new proteins would trigger a new challenge or new risks? More work perhaps as if we see with more cases! We find this argument outside what might be called a scientific argument. The guidelines are thus sufficient with a workload for applicant and risk assessors that are above proportionate to the expected (very low) risk? think about novel food plants and the many new proteins we can expect from them.	Comments noted. Regarding the new proteins, their individual assessment might not be manageable if a large number of proteins are to be assessed. Codex Alimentarius was mainly targeted for the assessment of few newly expressed proteins. More complex future products will challenge the overall practical implementation of such guidelines, mainly targeted to assess few newly expressed proteins. More challenging applications are expected in the future with large numbers of diverse proteins, for instance, derived from new genome techniques and synthetic biology. Therefore, it is timely to review and clarify the main purpose of the allergenicity risk assessment overall and the vital role it plays in protecting consumers' health with existing food allergies and assessing the potential for foods to cause new food allergies (EFSA GMO Panel, 2022).
77	3.2.2.3. Allergenicity	CropLife Europe	Line 492: Suggestion to replace "syn bio plant" with "case study 2". Lines 496-497: It is not clear which knowledge	Regarding the knowledge available from the SynBio design and modelling, the GMO Panel considers that it could effectively

			from modelling is being referred to as this example used a rather simple approach (standard bioinformatic analysis) which was applied to a plant with a complex genome.	anticipate the expected compositional characteristics of the SynBio product allowing the identification with reduced uncertainties of unintended effects.
78	3.2.2.3. Allergenicity	Testbiotech	In regard to allergenicity, both the intended and unintended effects have to be considered (see above). The draft text of EFSA is misleading in this respect when it states that no new proteins are expressed in these plants and that, therefore, no allergenic assessment of new proteins is needed. The wording has to be corrected to clarify that while no new proteins are expressed intentionally in the plants, allergenic assessment of potentially unintentionally produced new proteins (peptides) is needed. As argued above, each and every targeted genetic site needs to undergo a detailed examination to investigate whether the alpha-gliadin proteins are still being produced, or if new proteins are being unintentionally produced. There is no doubt that such unintended variations in genetic changes caused by New GE are associated with novel hazards and risks regarding allergenicity, even if no off-target genetic changes are identified. Furthermore, the EFSA assessment of allergenic risks in existing guidance is not based on sufficiently realistic exposure to newly introduced proteins and their interactions. Different routes of exposure, the timing of exposure, microbial exposure, oral and gut microbiota composition, epithelial barrier integrity and/or non-allergenic components of the food matrix, such as immune-modulating components (adjuvants) of allergenic sources that facilitate immune responses, have to be considered. However, the necessary methodology is not foreseen in existing risk assessment. Therefore, current guidance cannot be regarded as either adequate or sufficient.	Comment noted. The considerations proposed are not specific to Synbio GMPs, rather applicable to any GMPs. The current guidelines for the risk assessment of GMP for food and feed require to identify and characterise intended/unintended effects and to assess these as regards their safety and nutritional characteristics for humans and animals. In relation to allergenic risks in existing guidance, EFSA is actively engaged in evolving risk assessment methodologies based on the most recent scientific advancements (EFSA GMO Panel, 2022 on the need of developments in allergenicity; horizontal EFSA thematic grant GP/EFSA/ENCO/2020/02 – MICROBIOME). See also reply to comment 73.
79	3.2.2.4. Nutritional assessment	Euroseeds	Line 523-525: The total protein content in wheat varies a lot. Its content is an important consideration for all end products (uses of wheat) from bread baking to noodles, paste, cakes, and biscuits. Wheat protein content varies widely depending on wheat class, growing region, type and quality of soil, and of course fertilizers input (amount and timing), nitrogen in particular. While protein content is an intrinsic genetic trait and therefore a selection criterion in breeding programs, environmental impact is considerably greater than that controlled by the breeders gene pool (the breeders gene	Comment noted, general statement.

			pool includes sources of genes available for conventional plant breeding as outlined by EFSA in EFSA Journal 2012;10(2):2561). The guidelines for nutritional assessment might be adequate but in Euroseeds view not applicable due to the strong environmental influence on protein content in wheat independent of genetics (https://www.frontiersin.org/articles/10.3389/fpls.2016.00942/full).	
80	3.2.2.4. Nutritional assessment	Testbiotech	In the case of plants which are supposed to produce fewer proteins and may, therefore, have beneficial effects on health, both intended and unintended effects as well as their potential implications for overall food safety need to be taken into account. However, there is no appropriate methodology in place to assess associated specific intended or unintended health effects. Therefore, existing guidance for risk assessment cannot be regarded as adequate or sufficient.	Comment noted, general statement.
81	3.2.2.5. Dietary exposure	ANSES	No comment	Comment noted.
82	3.2.2.5. Dietary exposure	CropLife Europe	Line 529: Suggestion to replace "this SynBio wheat? with "gluten-free wheat" in line with the information provided in Table 2 and under section 3.2.2.4.	Comment noted, text edited.
83	3.2.2.6. Conclusions case study 2	ANSES	No comment	Comment noted.
84	3.2.2.6. Conclusions case study 2	BfN - Federal Agency for Nature Conservation	We do not agree with the conclusions. The existing guidance would require further developments as regards molecular characterisation, compositional analysis and toxicology, cf. comments on chapters 1.4, 3.1 and 3.2.2.2.	Comment noted. The adequacy of the molecular characterisation guidelines to this case study has been previously addressed (EFSA GMO Panel, 2021a).
85	3.2.2.6. Conclusions case study 2	Testbiotech	Existing EFSA guidance and Implementing Regulation 503/2013 are neither adequate nor sufficient to assess the risks of plants as described in Example 2.	Comment noted.
86	3.2.3. Case study 3 Fungal-resistant GM oilseed rape	ANSES	The interest of integrating these new gene and traits into the plant should be argued and justified by the petitioner. The development of this resistance will need to be demonstrated under cultivation conditions with controlled fungal infection.	Comment noted. The GMO regulatory frame requires that for any GMPs applicants provide a general description of the introduced trait(s) or modified information and its mode of action, of the resulting changes on the phenotype and the metabolism of the plant, and of its intended use. See also replies to comments 35, 61, 102.

87	3.2.3. Case study 3 Fungal-resistant GM oilseed rape	CropLife Europe	We note our disagreement with the designation of this case study as an example of application of synthetic biology. Insertion of a novel protein will not render a plant a SynBio, and even less so if it is a protein from a plant relative.	Comment noted. The specific Synbio GMP case study was selected because, even though it has been produced using existing GM (and therefore resemble current GMPs) its complexity is likely to require the application of SynBio approaches (see also EFSA GMO Panel, 2021a).
88	3.2.3.1. Comparative analysis	ANSES	The comparator should be precised. Logically, according the guidelines it should be the conventional counterpart as close as possible of the evaluated PGM. The evaluation of compositional analysis should be completed with the data of comparison for agronomic and phenotypic characteristics giving also information on possible modification of biological characteristics of rapeseed.	Comment noted. As indicated in your comment the ideal comparator is represented by the conventional counterpart of the evaluated SynBio oilseed rape.
89	3.2.3.1. Comparative analysis	CropLife Europe	Line 549: Suggestion to replace "SynBio oilseed rape" with "fungal-resistant GM oilseed rape" as in the introductory paragraph, and in line with the information in Table 2. Same change should be made throughout the document where "SynBio plant is used".	Comment noted.
90	3.2.3.1. Comparative analysis	BfN - Federal Agency for Nature Conservation	Case study 3 is described in Unkel (2020) which is the underlying report for EFSA (2021) as follows: "Case study 3: a fungal-resistant oilseed rape obtained by transgenic insertion of an existing plant resistance gene engineered to recognise a broader range of pathogens as well as the deletion of additional genes for pathogen susceptibility using genome editing." Neither the issue of unintended interactions from the several introduced and modified genes with the endogenous plant metabolism is raised here nor how to consider them within comparative analysis. As suggested in our comments on chapter 1.4 compositional analysis should be complemented by non-selective omics methods. Further, the risk assessment of GM plants expressing specific traits may require additonal treatment comparisons (EFSA 2011). In case of herbicide tolerance this relates to the application of intended, conventional or no herbicide(s), in case of fungal resistance this relates to the exposure towards different plant pests. As the natural presence of biotic stressors in field trials can vary tremendously, the requirements of the existing guidance in terms of the field trial design should be reconsidered and specified. This refers to the requirement to replicate each	Comment noted. Unintended interactions are addressed with the comparative analysis as in any other "conventional" GMP. The trait introduced in case study 3 is comparable with traits introduced with "conventional" GMP and the guidance on agronomic and phenotypic characterisation of GM plants (EFSA GMO Panel, 2015) remains valid also in the case study 3. The EFSA GMO Panel reminds that "*It is advisable […] to keep the treatments with pesticides to the minimum required to contain the level of disease/infestation below acceptable levels, since excessive use of plant protection products may impair a thorough evaluation of pathogen/pest–plant interactions. Similarly, the lack of pest management measures in the experimental field may lead to excessive stress on plants, which does not reflect normal agricultural practice.*" The GMO Panel also reminds that in accordance with EFSA (2015), in addition to generic agronomic endpoints, trait-specific ones can be selected if these are considered needed.

			field trial at a minimum of eight sites and the possibility to conduct it either in a single year, or spread over multiple years. The aim is to ensure that the fungal resistant OSR is exposed to a range of stressors singly and in combination during the comparative analysis of phenotypic and agronomic parameters. It should also be considered to additionally investigate the GMO in a greehouse where the exposure to different pathogens can be controlled. EFSA Panel on Genetically Modified Organisms (GMO) (2011). Guidance on selection of 767 comparators for the risk assessment of genetically modified plants and derived food and feeds. EFSA 768 Journal 2011;9(5):2149, 20 pp. doi:10.2903/j.efsa.2011.2149.	
91	3.2.3.2. Toxicology	ANSES	The comparative analysis will highlight the expression or production of new compounds or altered levels of constituents, needing toxicological assessment to assess potential unintended effects , in a 90-day toxicity study. The 90-day toxicity study in rodents allows testing the possible interactions between new proteins and the plant's original metabolic pathways within the matrix that will be consumed. The whole feed study on animals is also complementary.	Comment noted.
92	3.2.3.2. Toxicology	CropLife Europe	Line 559: Animal feeding studies should only be required if there is a testable risk hypothesis or hazard identified, regardless of how many new proteins are expressed, the animal welfare considerations remain. In case a hazard is identified, validated NAMs should be used to further assess the risk to humans/animals.	Comment noted.
93	3.2.3.2. Toxicology	BfN - Federal Agency for Nature Conservation	Line 564 - 567 We do not agree with this statement. According to Regulation (EC) 503/2013 a 90-day feeding study in rodents with whole food or feed is the primary additional study to address uncertainties identified in the course of the safety assessment. As the comparative analysis as well as the MC are based on preselected parameters/endpoints, non-selective methods to address unintended effects of the genetic modifications are missing and the conduction of a 90-day feeding study is justified.	Comment noted.
94	3.2.3.2. Toxicology	Testbiotech	In regard to toxicology, both the intended and unintended effects need to be considered. As argued above, each and every targeted genetic site needs to undergo a detailed examination to investigate whether new proteins are produced unintentionally, or if any other unintended effects	Comment noted. See replies to comments 73.

			may occur. Risk assessment also should take into account the emergence of other additional biological active molecules (such as ncRNAs) as well as interactions with the plants constituents. Furthermore, for example, impact on the immune system which can be effected via the intestinal microbiome has to be considered. However, existing guidelines for EFSA risk assessment, do not provide the methodology to comprehensively assess effects on the immune system, including chronic inflammation. Therefore, existing risk assessment cannot be regarded as adequate or sufficient.	
95	3.2.3.3. Allergenicity	ANSES	No comment	Comment noted.
96	3.2.3.3. Allergenicity	Testbiotech	EFSA assessment of allergenic risks in existing guidance is not based on sufficiently realistic exposure to newly introduced proteins and their interactions. Different routes of exposure, the timing of exposure, microbial exposure, oral and gut microbiota composition, epithelial barrier integrity and/or non-allergenic components of the food matrix, such as immune-modulating components (adjuvants) of allergenic sources that facilitate immune responses, have to be considered. However, the necessary methodology is not foreseen in existing risk assessment. Therefore, current guidance cannot be regarded as either adequate or sufficient.	EFSA is actively engaged in evolving risk assessment methodologies based on the most recent scientific advancements (EFSA GMO Panel, 2022 on development needs on allergenicity; EFSA thematic horizontal grant GP/EFSA/ENCO/2020/02 – MICROBIOME). EFSA has been proactive in this respect and has already invested resources to advance the allergenicity prediction further. A series of EFSA procurements have been undertaken, representing significant steps forward (Mills et al., 2013a,b; Mackie et al., 2019; Parenti et al., 2019; EFSA GMO Panel, 2017, 2021b). Likewise, EU-funded research programmes, such as the ImpARAS Cost Action, EuroPrevall, iFAAM and AllerScreening projects, among others, also provide insights for improvement of existing and suggested assessment tools in the field of allergenicity assessment of foods. However, significant knowledge gaps remain, and the development of novel approaches to deal with allergenicity assessment needs to be pursued further (EFSA, 2021; EFSA GMO Panel, 2022).
97	3.2.3.4. Nutritional assessment	ANSES	No comment	Comment noted.
98	3.2.3.5. Dietary exposure	ANSES	No comment	Comment noted.
99	3.2.3.6. Conclusions case study 3	ANSES	This case study appears very similar to a "conventional" PGM, as one trait is introduced.	Comment noted. The specific SynBio GMP case study was selected because, even though it has been produced using existing GM (and therefore resemble current GMPs) its complexity is likely to require the application of SynBio approaches (see also EFSA GMO Panel, 2021a).

100	3.2.3.6. Conclusions case study 3	BfN - Federal Agency for Nature Conservation	We do not agree with the conclusions. The existing guidance would require further developments as regards molecular characterisation, agronomic/phenotypic analysis, compositional analysis and toxicology, cf. comments on chapters 1.4, 3.1, 3.2.3.1 and 3.2.3.2.	Comment noted. The adequacy of the molecular characterisation guidelines to this case study has been previously addressed (EFSA GMO Panel, 2021a).
101	3.2.3.6. Conclusions case study 3	Testbiotech	Existing EFSA guidance and Implementing Regulation 503/2013 are neither adequate nor sufficient to assess the risks of plants as described in Example 3.	Comment noted.
102	3.2.4. Case study 4 De novo domesticated tomato	ANSES	The interest of integrating these new gene and traits into the plant should be argued and justified by the petitioner.	The GMO regulatory frame requires that for any GMPs applicants provide a general description of the introduced trait(s) or modified information and its mode of action, of the resulting changes on the phenotype and the metabolism of the plant, and of its intended use. See also replies to comments 35, 61, 85.
103	3.2.4. Case study 4 De novo domesticated tomato	Euroseeds	Line 606: Replace "this SynBio product (S. Pimpinellifolium)" and line 610 "SynBio tomato" with "this domesticated tomato"	Comment noted.
104	3.2.4. Case study 4 De novo domesticated tomato	BfN - Federal Agency for Nature Conservation	The draft does not consider to assess whether this new case study poses any new issues for the MC and thereby overlooks its role and function within the food and feed assessment. The de novo domesticated tomato is another example for the complexity of interventions with a likely range of alterations with the plant'" genome and metabolism. It is hardly conceivable that all of them can be predicted and addressed by targeted analysis. Therefore, non-selective screening methods (omics) need to be applied at genomic and phenotypic levels and the existing guidance adapted accordingly. There is hardly any experience with growing wild tomato compared to cultivated varieties. When the existing guidances was adopted, case study 4 was not on the table and the requirements for the field trial design and the range of phenotypic and agronomic characters were most likely meant for already cultivated crops. However, the de novo domesticated tomato - although modified - can still exhibit some wild characteristic. This should be considered by extending the range of receiving environments and the number of phenotypic and agronomic parameters.	This is a genome editing case study, technically covered in MC-ERA opinion where the conclusions of adequacy of GD requirements for RA of case study 2, are also applicable. As discussed in the different MC sections, some specific requirements referring to the introduced transgenes, would not be relevant or may need to be adapted for gene edited sequences. A general aspect related to the concepts used in some parts of the existing guidelines is that the technologies currently adopted as SynBio, are often based on altering the plant genome using gene editing instead of introducing a transgene. Therefore, in order to cover all technologies used, the term modification rather than transformation is more suited in the assessment of MC requirements while the concepts of event, junction site and flanking region may need to be reconsidered. In addition, one potential difference between traditional applications and future SynBio applications is the scale of the changes introduced.
105	3.2.4.1. Comparative analysis	Euroseeds	Lines 653-654- The conclusion of adequacy regarding comparative analysis is due to EFSA's narrow interpretation of what constitutes an appropriate comparator(s). We	Comment noted.

			support the suggestions made regarding alternative strategies, e.g., the use of multiple comparators reflecting the range of varieties commonly consumed. A more practical approach would be to facilitate discussions with developers during development and ensure the use of problem formulation, case-by-case and weight-of-evidence approaches so fit-for-purpose risk assessments can be prepared. We would like to refer to the Codex definition of conventional counterpart ("Conventional Counterpart" means a related organism/variety, its components and/or products for which there is experience of establishing safety based on common use as food) and to Regulation (EC) No 1829/2003 that defines a conventional counterpart as "a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use"(Art. 2.12). We note that the narrow interpretation of "appropriate comparator" as per existing EFSA guidance is a problem that is not unique to "Synbio GMPs" and needs to be addressed as commented above. This issue exemplifies the challenges of developing prescriptive guidance that does not provide adequate flexibility to conduct case-by-case risk assessment.	
106	3.2.4.1. Comparative analysis	ANSES	The evaluation of compositional analysis should be completed with the data of comparison for agronomic and phenotypic characteristics giving also information on possible modification of biological characteristics of tomato. The statistical analysis of difference will take into account data from two comparators. The experimental design for culture of PGM, comparators and conventional varieties will be slightly modified.	Possible unintended changes in agronomic and phenotypic characteristics on SynBio GMP are discussed in the previous Scientific Opinion (EFSA GMO Panel, 2021). The discussion covered the identified case studies as well as hypothetical more complex situations (see section 3.2.11). Those considerations remain valid also for the additional case study discussed in the current opinion.
107	3.2.4.1. Comparative analysis	CropLife Europe	Line 606: Suggestion to replace "this SynBio product (S. Pimpinellifolium)" and line 610 "SynBio tomato" with "this domesticated tomato" Lines 626-631: The statement made is not clear. Line 638: Multiplexing alone is not a synthetic biology approach, it is just application of genome editing to target different areas in one round. This could also be achieved in several rounds. However this technique is advantageous for plants with large and repetitive genomes, as in the case with tetra or hexaploid wheat. Lines 653-654: The conclusion of adequacy regarding comparative analysis	Comments noted.

			is due to EFSA's narrow interpretation of what constitutes an appropriate comparator(s). We support the suggestions made regarding alternative strategies, e.g., the use of multiple comparators. A more practical approach would be to facilitate discussions with developers during development and ensure the use of problem formulation, case-by-case and weight-of-evidence approaches so fit-for-purpose risk assessments can be prepared. We would like to refer to the Codex definition of conventional counterpart ("Conventional Counterpart" means a related organism/variety, its components and/or products for which there is experience of establishing safety based on common use as food) and to Regulation (EC) No 1829/2003 that defines a conventional counterpart as ?a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use"(Art. 2.12). We note that the narrow interpretation of "appropriate comparator" as per existing EFSA guidance is a problem that is not unique to "Synbio GMPs" and needs to be addressed as commented above. This issue exemplifies the challenges of developing prescriptive guidance that does not provide adequate flexibility to conduct case-by-case risk assessment.	
108	3.2.4.1. Comparative analysis	BfN - Federal Agency for Nature Conservation	Line 632 - 636 The existing guidelines allow to use additional comparators for certain cases to carry out a comparative assessment. The proposed extension of this concept, i.e. the use of multiple comparators, seems to stretch the concept too far. Therefore, we support the more obvious solution of the guidance that where no appropriate conventional counterpart can be identified, a comparative safety assessment cannot be made and a safety and nutritional assessment of the GM food and feed shall be carried out as for novel foods.	Comment noted.
109	3.2.4.1. Comparative analysis	Testbiotech	Example 4 shows that the complexity of the newly introduced CRISPR/Cas-induced genetic changes results in a new quality of hazards and risks (see also Kawall, 2021). However, neither the molecular characterisation in the previous EFSA report (EFSA 2021) nor the provided text for consultation reflect recent scientific findings. Therefore, under example two, we attach a short update on molecular characterisation of SynBio-Plants which, in most of its topics, is of relevance also for example 4 (see Annex). More	Comment noted; please see response to comment 67.

			specifically, in regard to example 4, in de novo domestication, CRISPR/Cas9 is used to alter the genomes of wild species in such a way that some of their genes are modified to resemble domesticated ones. Such de novo domesticated plants still have some properties from wild species that have been lost during plant breeding. As also shown by the draft oponion of EFSA (2022), plants altered with SDN-1 which contain traits that are known from cultivated varieties, but are now expressed in a new genetic background, cannot be equated to their conventional or natural counterparts, as the corresponding target gene(s) might have divergent functions or interactions in different species (see Kawall, 2021). The problems with the identification of the adequate comparators are relevant for many of plants derived from NGT (SynBio methods) and therefore, new guidelines will be needed how to conduct risk assessment per se (stand alone risk asessessment), while the exiting comparative approach can not be seen as sufficient. There is thus a need for comprehensive methodology to assess changes in plant composition and phenotypic characteristics, which also makes use of "omics" (genomics, transcriptomics, proteomics, metabolomics). In addition, the plants should be exposed to a sufficiently broad range of biotic and abiotic stressors to investigate the extent to which these factors impact plant composition, phenotypic characteristics and gene expression.	
110	3.2.4.2. Toxicology	Federal Office of Consumer Protection and Food Safety (BVL) (National Authority)	The sentence is unclear in the part "except for the newly expressed proteins, when the guidelines are not applicable" as no proteins are newly expressed in this case study. It is suggested to rephrase to "except for the provisions on newly expressed proteins, when the guidelines are not applicable"	Comments noted, text edited as needed. See reply to comment 73.
111	3.2.4.2. Toxicology	ANSES	The 90-day toxicity study in rodents allows testing the possible interactions between new proteins and the plant's original metabolic pathways within the matrix that will be consumed. The whole feed study on animals is also complementary. L664 : I suggest to delete this sentence, as there are no new proteins: "except for the new proteins, for which they are not applicable ".	
112	3.2.4.2. Toxicology	CropLife Europe	Line 656: Suggestion to replace ?SynBio plant? with Case study 4.	Comment noted.

113	3.2.4.2. Toxicology	BfN - Federal Agency for Nature Conservation	Line 656 - 657 The interventions and alterations at the genomic level are complex and the expression of new proteins due to e.g. frame shift mutations has to be considered. This can best be assessed by applying different omics method and the existing guidance should be supplemented accordingly. Line 660 - 661 We do not agree with this statement. According to Regulation (EC) 503/2013 a 90-day feeding study in rodents with whole food or feed is the primary additional study to address uncertainties identified in the course of the safety assessment. As the comparative analysis as well as the MC are based on preselected parameters/endpoints, non-selective methods to address unintended effects of the genetic modifications are missing and the conduction of a 90-day feeding study is justified.	Comments noted.
114	3.2.4.2. Toxicology	Testbiotech	In regard to toxicology, both the intended and unintended effects have to be considered. The draft text of EFSA is misleading in this respect when it states that no new proteins are expressed in these plants and that, therefore, no toxicological assessment of new proteins is needed. The wording has to be corrected to clarify that, while no new proteins are expressed intentionally in these plants, toxicological assessment of potentially unintentionally produced new proteins (peptides) is needed. As argued above, each and every targeted genetic site needs to undergo a detailed examination to investigate whether new proteins are produced unintentionally, or if any other unintended effects may occur. Risk assessment also should take into account the emergence of other additional biological active molecules (such as ncRNAs) as well as interactions with the plants constituents. It should be fully taken into account that the intended as well as the unintended variations of genetic change caused by New GE are associated with novel hazards and risks regarding toxicology, even if no off-target genetic changes are identified. Furthermore, for example, impact on the immune system which can be effected via the intestinal microbiome has to be considered. However, existing guidelines for EFSA risk assessment, do not provide the methodology to comprehensively assess effects on the immune system,	Comment noted. See reply to comment 73.

			including chronic inflammation. Therefore, existing risk assessment cannot be regarded as adequate or sufficient.	
115	3.2.4.3. Allergenicity	Federal Office of Consumer Protection and Food Safety (BVL) (National Authority)	The sentence is unclear in the part "except for the newly expressed proteins, when the guidelines are not applicable" as no proteins are newly expressed in this case study. It is suggested to rephrase to "except for the provisions on newly expressed proteins, when the guidelines are not applicable"	Comment noted, text edited.
116	3.2.4.3. Allergenicity	ANSES	We have to keep in mind that allergenicity of fruits and, especially, of tomato, depends on both the pulp and seed allergenic components. In this respect, tomato seeds contain cupin allergens (7S vicilin and 11S legumin) which could be responsible for allergic reactions in previously sensitized people.	Comment noted.
117	3.2.4.3. Allergenicity	CropLife Europe	Line 666: Suggestion to replace "This SynBio plant" with "The plant in Case study 4".	Comment noted, text edited.
118	3.2.4.3. Allergenicity	Testbiotech	EFSA assessment of allergenic risks in existing guidance is not based on sufficiently realistic exposure to newly introduced proteins and their interactions. Different routes of exposure, the timing of exposure, microbial exposure, oral and gut microbiota composition, epithelial barrier integrity and/or non-allergenic components of the food matrix, such as immune-modulating components (adjuvants) of allergenic sources that facilitate immune responses, have to be considered. However, the necessary methodology is not foreseen in existing risk assessment. Therefore, current guidance cannot be regarded as either adequate or sufficient.	EFSA has been proactive in this respect and has already invested resources to advance the allergenicity prediction further. A series of EFSA procurements have been undertaken, representing significant steps forward (Mills et al., 2013a,b; Mackie et al., 2019; Parenti et al., 2019; EFSA GMO Panel, 2017, 2021). Likewise, EU-funded research programmes, such as the ImpARAS Cost Action, EuroPrevall, iFAAM and AllerScreening projects, among others, also provide insights for improvement of existing and suggested assessment tools in the field of allergenicity assessment of foods. However, significant knowledge gaps remain, and the development of novel approaches to deal with allergenicity assessment needs to be pursued further (EFSA, 2021; EFSA GMO Panel, 2022).
119	3.2.4.4. Nutritional assessment	ANSES	No comment	Comment noted.
120	3.2.4.4. Nutritional assessment	CropLife Europe	Line 681: We do not agree that this constitutes a SynBio case study. Suggestion to delete "synBio".	Comment noted. The domesticated tomato was chosen as its complexity is likely to require the application of SynBio approaches.
121	3.2.4.5. Dietary exposure	ANSES	No comment	Comment noted.
122	3.2.4.5. Dietary exposure	CropLife Europe	Line 683: Suggestion to replace "this SynBio plant" with "case study 4".	Comment noted, text edited.
123	3.2.4.6. Conclusions case study 4	ANSES	No comment	Comment noted.

124	3.2.4.6. Conclusions case study 4	CropLife Europe	Line 689: This is not a SynBio case study, as such. Suggestion to replace "SynBio" by "hypothetical" . Line 703: Suggestion to insert "selected hypothetical case studies".	Comments noted. The domesticated tomato was chosen as its complexity is likely to require the application of SynBio approaches.The selected case studies (including this one) are described as hypothetical throughout the opinion.
125	3.2.4.6. Conclusions case study 4	Euroseeds	Line 689: This is not a SynBio case study, as such please replace "SynBio" by "hypothetical" Line 703: insert "selected hypothetical case studies".	Comments noted. The domesticated tomato was chosen as its complexity is likely to require the application of SynBio approaches. The selected case studies (including this one) are described as hypothetical throughout the opinion.
126	3.2.4.6. Conclusions case study 4	BfN - Federal Agency for Nature Conservation	We do not agree with the conclusions. The existing guidance would require further developments as regards molecular characterisation, agronomic/phenotypic analysis, compositional analysis and toxicology, cf. comments on chapters 1.4, 3.1, 3.2.4, 3.2.4.1 and 3.2.4.2.	Comment noted. The GMO Panel agrees that further development is needed as regard to comparative compositional analysis that implies considerations also on the general principles of comparative analysis laid down in EFSA GMO Panel, 2011 and 2015.
127	3.2.4.6. Conclusions case study 4	Testbiotech	Existing EFSA guidance and Implementing Regulation 503/2013 are neither adequate nor sufficient to assess the risks of plants described in Example 4.	Comment noted.
128	4.1. Conclusions on ToR1	Federal Office of Consumer Protection and Food Safety (BVL) (National Authority)	The German Federal Office for Consumer Protection and Food Safety (BVL) supports the conclusion, that no new sectors/advances exceeding the already identified the six SynBio categories are identifiable.	Comment noted.
129	4.1. Conclusions on ToR1	BfN - Federal Agency for Nature Conservation	We agree with Unkel (2020), who remark that many scientists would not consider this kind of work on metabolic engineering as being synthetic biology. This particularly refers to case studies 2 and 4 where complex modifications without transgene insertion were reached using multiplexed gene editing. Both cases should have been dealt with in EFSA (2020). We do not share the conclusions of EFSA (2021), cf. our comments on this document and here on chapter 1.4. The potential of genome editing for new and complex changes as well as the potential for unintended effects at the molecular level (on-target effects) as described in the recent literature has been overlooked or not been taken into consideration. EFSA Panel on Genetically Modified Organisms (GMO) (2020): 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. In: EFSA Journal 18 (11), S. 1611. DOI: 10.2903/j.efsa.2020.6299. EFSA Panel on Genetically Modified Organisms (GMO) (2021): Evaluation of existing guidelines for their adequacy for the molecular characterisation and environmental risk	Comment noted. See reply to comment 20. In its scientific opinion on SDN-1, SDN-2 and oligonucleotide directed mutagenesis (ODM), EFSA concluded that there are no new hazards specifically linked with these techniques, compared to conventional breeding. Although the multiplexing approach is not specifically discussed in the opinion, the GMO Panel has addressed this issue in the context of the relevant public consultation (EFSA GMO Panel, 2020). The GMO Panel has concluded that all the considerations in the opinion are also applicable to multiplexing approaches. It has also noted that multiplexing is not specific to SDN/ODM approaches, as it can also be achieved by transgenic and conventional breeding approaches. Classical breeding techniques (e.g. marker assisted selection) allow also the association of multiple traits (mutations) in a given variety. The GMO panel has recognised that this simultaneous association can be achieved potentially more rapidly using SDN-based techniques and this aspect has been taken into consideration in the opinion. The GMO Panel has also acknowledged that the application of SDN-based methods can lead to a complexity of scenarios and that the "case-by-case" approach is applicable to genome edited plants.

			assessment of genetically modified plants obtained through synthetic biology. In: EFSA Journal 19 (2). DOI: 10.2903/j.efsa.2021.6301. Unkel, K; Krause, D; Sprink, Thorben; Hartung, Frank; Wilhelm, Ralf (2020): Mapping of plant SynBio developments in the agri?food sector. In: EFS3 17 (3), S. 287. DOI: 10.2903/sp.efsa.2020.EN-1687.	
130	4.1. Conclusions on ToR1	Testbiotech	Previous conclusions from EFSA in 2021 are not applicable. They do not not address correctly nor sufficiently the differences of SynBio-Plants (NGT plants) in comparison to (previous methods used and results derived from) conventional breeding and genetic engineering sufficiently (see also Annex).	Comment noted.
131	4.2. Conclusions on ToR2	Federal Office of Consumer Protection and Food Safety (BVL) (National Authority)	The German Federal Office for Consumer Protection and Food Safety (BVL) supports the conclusion, that no potential hazards and risks for humans and animals that could be posed by food and feed from GM plants obtained through current and near future SynBio approaches were identified.	Comment noted.
132	4.2. Conclusions on ToR2	CropLife Europe	Line 699: Suggestion to insert "On the basis of the evaluation of selected hypothetical case studies".	Comment noted. The hypothetical nature of the selected case studies is well highlighted throughout the opinion.
133	4.2. Conclusions on ToR2	BfN - Federal Agency for Nature Conservation	We do not agree with the conclusions. SynBio plants are likely more complex with several new traits, a high number of new proteins without HOSU and more and complex interventions at the molecular level and complex interactions at the metabolic level, leading to numerous new compounds/altered levels of constituents which could pose new hazards and risks to human and animal health.	Comment noted.
134	4.2. Conclusions on ToR2	Testbiotech	The EFSA conclusion (EFSA, 2022) that, based on the four examples given above, no novel potential hazards and risks for humans and animals can be identified, is not sufficiently supported by scientific findings and stands in contradiction to recent publications, which EFSA either overlooked or ignored. Much more, intended and unintended effects associated with New GE techniques (or what is called SynBio approaches), such as the application of gene scissors like CRISPR/Cas9, elevate the hazards and risks to a level far beyond any assessment thus far. Genome editing has the unprecedented power to make large parts of the genome accessible to change, by overriding the natural mechanisms of genome organization such as repair mechanisms or backup genes. Thereby, New GE techniques can cause	Comment noted. Please see response in comment 67.

			pervasive changes in the genome of plants and animals, without inserting additional "foreign" genes. These processes are also known to result in unintended effects, especially if "gene scissors" (site directed nucleases or SDNs) such as CRISPR/Cas are applied. Both intended and unintended genetic changes can go far beyond what was seen in applications of previous methods. Some potential intended and unintended effects are specific to the techniques of New GE and may result in a new quality of risks that demand independent and mandatory risk assessment.	
135	4.3. Conclusions on ToR3	Federal Office of Consumer Protection and Food Safety (BVL) (National Authority)	The German Federal Office for Consumer Protection and Food Safety (BVL) supports the conclusion, that the existing guidelines are in principle adequate and can therefore be applied for the food and feed risk assessment of GM plants obtained through SynBio approaches that are likely to be marketed in the close future. The BVL also agrees, that in some cases updates and evolution of existing guidelines may be supportive for effective and safe risk assessments.	Comment noted.
136	4.3. Conclusions on ToR3	CropLife Europe	Line 706-707: Suggestion to add in, or after this sentence a comment that in some cases data requirements do not apply.	Comment noted.
137	4.3. Conclusions on ToR3	BfN - Federal Agency for Nature Conservation	We do not share the conclusion that the existing guidelines are in principle adequate; they need some substantial update in terms of molecular characterisation, agronomic/phenotypic analysis, compositional analysis and toxicology. Also, based on recent scientific findings, it cannot a priori be assumed that certain NGT plants would occur naturally or through conventional breeding (EFSA 2020). Therefore, the case-by-case assessment needs to be reinforced (Eckerstorfer et al. 2021, Kawall 2021). EFSA Panel on Genetically Modified Organisms (GMO) (2020): 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. In: EFSA Journal 18 (11), S. 1611. DOI: 10.2903/j.efsa.2020.6299. Eckerstorfer, Michael F.; Grabowski, Marcin; Lener, Matteo; Engelhard, Margret; Simon, Samson; Dolezel, Marion et al. (2021): Biosafety of Genome Editing Applications in Plant Breeding: Considerations for a Focused Case-Specific Risk Assessment	Comment noted. Please see response to comment 67.

			in the EU. In: BioTech 10 (10). DOI: 10.3390/biotech10030010. Kawall, Katharina (2021): The Generic Risks and the Potential of SDN-1 Applications in Crop Plants. In: Plants (Basel, Switzerland) 10 (11), S. 2259. DOI: 10.3390/plants10112259.	
138	4.3. Conclusions on ToR3	Testbiotech	While the EU legal framework might in general be seen to offer adequate regulatory provisions, the existing guidance and Implementing Regulation 503/2013 cannot be regarded as adequate or sufficient to comprehensively address these risks. There is increasing evidence that factors intrinsic to New GE techniques deserve much more attention by the regulators. For example, according to Yang et al. (2022), "mutation locations and scales, potential off-target modifications, complexity of the introduced changes, and novelty of the developed traits" make it necessary to apply "rigorous research on genome-editing applications and reliable techniques for risk assessments of genome-edited plants". Kawall (2021) in investigating the generic risks associated with the application of the CRISPR/Cas machinery concludes: "In summary, this review here shows that about half of the market-oriented plants developed by SDN-1 applications contain complex alterations in their genome (i.e., altering multiple gene variants or using multiplexing). It also illustrates that data on both the process- and the end-product are needed for a case-by-case risk assessment of genome edited plants. The broad range of genetic alterations and their corresponding traits reflects how diverse and complex the requirements are for such a risk assessment." Eckerstorfer et al. (2021) come to similar conclusions: "Based on these considerations, further guidance should be developed to ensure the high safety standards provided by the current regulatory framework for GMOs in the EU for GE plants in an adequate and efficient way, taking into account the existing knowledge and experience in a case-specific manner. This guidance should thus strengthen the case-specific approach that is recommended by numerous EU and Member States institutions."	Comment noted, please see response in comment 67.
139	4.4. Conclusions on ToR4	Federal Office of Consumer Protection and Food Safety (BVL) (National Authority)	The German Federal Office for Consumer Protection and Food Safety (BVL) supports the conclusion, that certain aspects of risk assessment may benefit from an evolution	Comment noted.

			of guidelines to accommodate for aspects resulting from complex traits GMP obtained by new molecular technologies. The suggestion to integrate alternative risk assessment approaches for the safety and nutritional assessment as for other novel foods is also supported.	
140	4.4. Conclusions on ToR4	CropLife Europe	Lines 711-713: We agree that evolution of current testing methods is warranted but we do not agree that this driven by the number of proteins to be tested. We note that this is a necessary development (also in line with the EU 3R legislation), irrespective of the number of proteins to be analyzed. Lines 715-722: We note that the narrow interpretation of what constitutes "comparative assessment" and "appropriate comparator" is in the heart of the challenges identified by the GM panel and it underlines the problem with overly prescriptive guidance. - We recommend that a note is inserted to recognize the general need for flexible risk assessment approaches and that this is best achieved though case-by-case risk assessments, using problem formulation and credible hypothesis for a pathway to harm to determine the relevant data requirements. Developing additional prescriptive guidance is unlikely to provide the needed flexibility to address future developments in genetic engineering of plants. Line 728: Please insert the specific article or section of Regulation (EU) No 503/2013 that substantiates this bullet.	Comments noted.
141	4.4. Conclusions on ToR4	Euroseeds	Line 724: "the choice of the comparator and the identification of multiple comparators;" Euroseeds would like to highlight that the variation of constituents and with that the composition of foods within the breeders' gene pool can be huge. Specifically, when it comes to SynBio GMPs that do not express novel proteins (e.g. case study 2/4) the adequate comparator would be the diversity as reflected in the breeders gene pool. So the trigger for any form of risk assessment should be linked to compositional changes that go beyond those which can be achieved by the diversity as reflected in the breeders gene pool in form of varieties that are commonly consumed. Lines 711-713: Future SynBio developments can go in many directions, instead of adjusting the guidelines that may not address every new SynBio application, the risk assessment should be case by case process with enough flexibility to apply appropriate	Comments noted. Multiple comparators to capture the diversity. The GMO Panel has assessed the possibility to evaluate GMPs Synbio in the context of the current legal frame. In the European Union, the statistical evaluation of the comparative assessment requires the simultaneous application of two complementary tests: a test of difference, to identify possible differences between the GM plant and its appropriately selected non-GM conventional counterpart with a history of safe use (OECD, 1993); and a test of equivalence to assess whether the characteristics of the GM plant fall within the range of natural variation estimated from a set of conventional non-GM reference varieties with a history of safe use (EFSA GMO Panel 2011a.; van der Voet et al., 2011). The non-GM reference varieties are selected by the applicant to reflect the diversity in the varieties that are commonly consumed. Additional comparators can be included in the field trial design (i.e. null-segregant, conventional GM reference varieties etc.) to

			requirements. This could be achieved through dialogue with applicants since development stages and pre-submission meetings where approach and applicability of the requirements for the specific SynBio product is discussed.	support the interpretation of possible differences and or lack of equivalence.
142	4.4. Conclusions on ToR4	German Central Committte on Biological Safety (ZKBS)	The report concludes that existing guidelines may need updating where the safety of new proteins is to be assessed, also regarding the comparative/compositional analysis. This means that novel traits of the plants will have to be assessed, but not the individual approaches and subsequent techniques used for their creation. In consistency to the document on microorganisms ("As the technique-driven risk assessment has his limitations, especially for the assessment of SynBioM, a strain-driven approach can be envisaged for all future SynBioM assessments."), the ZKBS encourages the EFSA to also apply a product-driven rather than a process-driven or technique-based risk assessment approach.	Comment noted.
143	4.4. Conclusions on ToR4	BfN - Federal Agency for Nature Conservation	The existing guidance needs coniserable update to integrate screening methods at different organizational levels into the risk assessment and " because of the absence of suitable comparators " to develop a stand alone assessment.	Comment noted.
144	4.4. Conclusions on ToR4	Testbiotech	Areas where the existing guidelines will need updating and further guidance is needed include those related to the molecular characteristics, safety assessment of new proteins and any other potentially biological active molecules such as ncRNAs (produced intentionally or unintentionally), the comparative/ compositional analysis, the analysis of intended and unintended agronomic and phenotypical characteristics, toxicology (also taking into account non-IGE immune reactions and the role of the microbiome), mixed toxicity, allergenicity and nutritional assessment. In addition, guidance is needed how to address combinatorial effects and introduce effective post marketing monitoring. In fact, the uncertainties about safety of EU food and feed production have been increasing since the first GE plants were introduced. In addition, environmental damage is being caused in the producing countries.. These systemic problems are likely to increase strongly if SynBio plants are introduced at large scale, big numbers, including many traits and different species into the environment and the food system. Further guidance should also take into account systemic effects by applying prosp5ective technology assessment and	Comment noted.

			horizon screening. Such a guidance should also address how to integrate scenarios that include potential impacts and interactions of accumulated and combinatorial effects caused by the presence of more than one SynBio plant in the food chain. One starting point to address thes issues should be a further case study in the opinion of EFSA which is elaborating on risk assessment of a diet which is mixture of the four SynBio plants as described in case study 1-4.	
145	4.5. Recommendations	ANSES	The evaluation of compositional analysis should be completed with the data of comparison for agronomic and phenotypic characteristics giving also information on possible modification of biological characteristics of PGM Synbio and selected comparator(s). Developing new approaches such as in silico prediction tools and in vitro testing for protein toxicity and mixtures toxic effects is needed. It is relevant to consider complex Synbio PGM and derived food/feed as novel foods, for alternative risk assessment approaches for the safety and nutritional assessment .	Comment noted.
146	4.5. Recommendations	CropLife Europe	Lines 732-735: The scope of the draft opinion is on food/feed risk assessment. This recommendation seems to be out of the scope since it focuses on the research and development phase. We understand that R&D phase of a specific product is under the developer' responsibility and it's not under EFSA's remit. Therefore it does not seem appropriate to include such recommendations in a potential future guidance document.	Comment noted.
147	4.5. Recommendations	Testbiotech	In view of plans to introduce, without any kind of precedent, a large number of SynBio plants with different traits and from many species into the environment and into the food and feed chain, which inherit biological characteristics far beyond what was achieved with previous methods, a concerted effort is needed to develop internationally agreed guidance and harmonised frameworks to strengthen the precautionary principle. Decision making over the use of genetic engineering and the introduction of plants and animals derived from old or new techniques of genetic engineering, therefore, has to be guided by the precautionary principle, to prevent ecosystems and food systems from being flooded with too many risks, uncertainties and unknowns within a short period of time	Comment noted.

			(and expanding over time). As with the need to reduce the use of plastics and toxics such as pesticides, there is a need to restrict the introduction of organisms with human-made genetic design into the environment and the food production systems. Problems created by the introduction of SynBio plants may last as long as, or longer than, those from plastics and pesticides, with impacts on many future generations. Consequently, not only the risks associated with individual GE organisms, but also the systemic risks and potentially disruptive effects of using New GE need to be taken into account. Therefore a comprehensive and prospective technology assessment has to be conducted to address systemic risks. This is especially relevant if, within a short period of time, many of these genetically engineered organisms are introduced into the environment, agro-ecosystems and food systems. If these findings overlooked in regulation, the introduction of New GE organisms (or SynBio plants) will endanger ecosystems and food production	
149	References	Federal Office of Consumer Protection and Food Safety (BVL) (National Authority)	Line No. 801: The link to the doi-number provided is compromised by an excessive space	Comment noted.
150	References	CropLife Europe	Line 778: For consistency across the Reference list we would recommend to use "EFSA GMO Panel (EFSA)".	Comment noted.
151	References	Testbiotech	see annex as uploaded to example 2	Comment noted.
152	blank	National Food Institute, Technical University of Denmark		Comment noted.

3. References (EFSA comments)

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Glossary and Abbreviations

Glossary: an alphabetical list of words relating to a specific subject, text, or dialect, with explanations; a brief dictionary.

Abbreviation: a shortened form of a word or phrase (such as Mr., Prof.). It also includes acronyms (a group of initial letters used as an abbreviation for a name or expression, each letter being pronounced separately – such as DVD, FDA – or as a single word – such as EFSA, NATO).

ANSES	French agency for Food, Environmental and Occupational Health & Safety
BfN	Federal Agency for Nature Conservation
BVL	Federal Office of Consumer protection and Food Safety
ZKBS	German Central Committee on Biological Safety

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

Scope of Consultation

EFSA's Methodology and Scientific Support (MESE) Unit has launched an open consultation on a draft scientific opinion from the Scientific Committee regarding microorganisms developed through synthetic biology. In line with the mandate of the European Commission, the opinion provides an evaluation of the adequacy of existing guidelines for the risk assessment of food and feed from genetically modified microorganisms obtained through synthetic biology. For context and other work from EFSA on New Advances in Biotechnology, including prior opinions on synthetic biology (molecular characterization and environmental risk assessment aspects), please consult https://www.efsa.europa.eu/en/topics/topic/new-advances-biotechnology

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

Additional data or files to support the comments may be submitted using the relevant function in the digital form.

All comments will be considered, so long as they:

- are submitted by the closing date of the consultation;
- are finalised (comments in 'draft' status will not be accepted);
- are presented according to the instructions and relevant function in the tool (regrettably, we cannot accept comments sent by email);

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

Copyright-cleared contributions:

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

Publication of contributions:

Third-party comments will be made public in their original form without delay after the closing date of the consultation and may be reused by EFSA in a different context. The outcome of the consultation will be made public in conjunction with the publication of the relevant scientific output. Contributions submitted by individuals in a personal capacity will be published indicating the author's first and family name unless the respondent has requested anonymity.

Contributions submitted on behalf of an organisation will be attributed to the organization in question.

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

Appendix B – Comments submitted in separated files

B.1. German Central Committee on Biological Safety (ZKBS): Comment to 2.2 Selection of case studies to address WP4

There is no clear definition for synthetic biology, which makes it difficult to identify case studies. This is well illustrated by the scientific committees´ (SCENIHR, SCCS, and SCHER) definition that defines synthetic biology as "the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in viable organisms". This definition does not allow, or at least is not sufficient, to differentiate between "classical" genetic engineering and synthetic biology. In the EFSA document it is stated that it is not the technique used, but the technological complexity of the approach that qualifies a case study as being synthetic biology.

The ZKBS considers the case studies chosen in this report as classical genetic engineering (transgenesis) or targeted mutagenesis applications as SDN-1. In addition, all chosen case studies are not considered "complex" which would justify classifying them as synthetic biology. The plants are modified by either inserting heterologous genes/stacks or by introducing targeted mutations via CRISPR/Cas. Substantial attributes of synthetic biology such as "the further development of molecular biological methods enabling significantly more extensive manipulations, the large-scale use of bioinformatics enabling a modelled approach and the efforts to enhance the predictability of these manipulations via standardised components" (see Monitoring of Synthetic Biology in Germany, ZKBS 2012, available at: https://tinyurl.com/2nmd5wad) cannot be identified. An example to underline the above written is the case study 2. Two sgRNAs were designed to target a conserved region in the α-gliadin genes which results in a strong reduction in α-gliadins. The use of two sgRNAs in genome editing does not lead to an engineering of "complex, quantitative traits controlled by multiple genes (e.g. photosynthetic capacity and nutrient use efficiency); for the design of traits that require lengthy multigene pathways (e.g. to produce new metabolites); and for the de novo design of proteins able to perform new or expanded functions" (EFSA 2021, EFSA J 19(2):e06301). On the contrary, the genome editing approach in case study 2 resulted in point mutations, which also could be obtained by conventionally breeding or occur naturally which is not the case for products of SynBio applications. Since the examples chosen are classical GMO approaches, it is a good confirmation that the existing directives are appropriate for evaluating GMOs. Since synthetic biology organisms are generally regarded as GMOs it is expected that existing directives would also apply to more sophistically engineered plants. Instead of adjusting the guidelines to all potential future SynBio the risk assessment should follow a case-by-case process with enough flexibility to apply appropriate requirements.

B.2. CropLife Europe General Comments Draft EFSA Scientific Opinion on "Evaluation of existing guidelines for their adequacy for the food and feed risk assessment of genetically modified plants obtained through synthetic biology" 11 March 2022

CropLife Europe welcomes the opportunity to comment on this document, regarding the adequacy of the current risk assessment framework for GMPs in the EU to assess plants modified using synthetic biology approaches. CropLife Europe notes that this exercise represents an opportunity to capitalise on the wealth of information and experience gained on the risk assessment for GMPs, to ensure that proportionate and fit-for purpose assessments are conducted for regulated products, including these developed with the use of recent scientific and technological advances. The Scientific Opinion describes the current approach for conducting a food and feed risk assessment of genetically modified plants (GMPs) through application to four hypothetical "synthetic biology" case studies. Our industry has commented previously in our submission on the 2021 EFSA Scientific Opinion on Synthetic Biology Developments in Plants, MC and ERA, that the three first case studies considered in the EFSA document are not representative of synthetic biology GMPs, but pertain to transgenesis (referred to as "classical GMPs" in the document) or to gene-edited plants. Therefore CropLife Europe does not agree with the use of the term "SynBio GMPs" to refer to the case studies considered in this opinion. We are also of the view that Case study 4, newly included in the current opinion is also not an example of "synthetic

biology". The consideration that the use of information technology and state of the art know-how for plant breeding results in yet another loosely defined technology category of "synthetic biology" is concerning and is likely to create additional hurdles and disincentives for developers on top of these that are associated with the use of biotechnologies in the plant sciences and in breeding. The Scientific Opinion makes an assessment as to the applicability, sufficiency (fully fit for purpose), or adequacy (need for additional qualifications) of existing guidance documents. CropLife Europe agrees with the conclusions of the GMO Panel who do not identify novel potential hazard and risk for humans and animals from GM plants obtained through synthetic biology approaches, and with their assessment that existing guidance documents are sufficient for most elements of the risk assessment. While we believe the current approaches to risk assessments for GMPs outdated and not reflecting the experience of more than 25 years of food/feed risk assessment, we support the conclusion that no additional guidance is required but rather that problem formulation should be introduced in all steps of the risk assessment to ensure that only relevant data points are assessed and to avoid disproportionate data requirements. We also support the conclusions that existing guidance documents are not applicable in all cases (e.g. where no new proteins are expressed) and consider that this case-by-case flexibility should be the default approach for every GMP risk assessment, independent of the technology used, or the resulting product, and product classification. Data requirements should be consistent with the fundamental principle of case-by-case approach and fit for purpose risk assessment based on problem formulation. As such, and aligned with our previous submission for the 2021 EFSA Scientific Opinion we recommend that the report reflects the following points: • Performing case-by-case risk assessments, using problem formulation to determine the relevant data requirements for every regulated GMP, including future "SynBio GMPs". Requests for data should be based on a credible hypothesis for a pathway to harm. • Support for derogation from some specific data requirements outlined in CIR 503/2013, in line with the principle for case-by-case risk assessment, that may not be relevant for particular regulated plant and only lead to unnecessary animal testing (e.g., the 90 days study when there is no plausible risk hypothesis from the comparative analysis). • Facilitation of early dialogue between risk assessors and product developers (e.g. presubmission consultations) as an efficient way to ensure fit-for-purpose risk assessments. • Underline the importance of flexibility for implementation of guidance on risk assessment and that the generation of over-prescriptive guidance documents limits the implementation of the case-by-case approach

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B.4. TESTBIOTECH Background 20 - 3 - 2022 Updated review on risk assessment and molecular characterisation of plants derived from new genomic techniques (NGT, SynBio, New GE, genome editing) Prepared as annex for consultation on SynBio plants (EFSA 2022)

For the first time, genome editing makes large parts of the genome of many species accessible to change (via targeted mutations) (Kawall 2019). The CRISPR/Cas techniques can override the natural mechanisms in genome organisation that protect essential genes (Belfield et al., 2018; Frigola et al., 2017; Halstead et al., 2020; Kawall, 2019; Monroe et al., 2022). As a result, novel genotypes and biological characteristics can emerge from applications of this technology. These observations are relevant to both intended and unintended effects. Tools such as CRISPR/Cas can prevent the cells from restoring the original function of the gene (Brinkmann et al., 2018); they can also override other natural protection mechanisms (for overview, see Kawall, 2019). In addition, CRISPR/Cas can also block the function of all the 'backup' copies of a target gene, of which there can be several in the genome of the plant. In this context, it is not the number of changes per se which has to be taken into account, but the patterns of change as well as the genotypes and phenotypes resulting from these intended and unintended changes (see also Kawall, 2021). At each stage of the process, such as (i) insertion of the DNA of the gene scissors into the cells, (ii) target gene recognition and cutting and (iii) cellular repair of the genes, specific unintended changes can occur, with associated risks (for overview, also see Kawall et al., 2020). For example, changes caused by the non-targeted insertion of transgenic elements in the first step of the process may remain in the plants and impact safety, even if the transgenic elements are removed by further breeding at the end of the process. In this context, there are a number of publications reporting unintended effects arising from the application of 'Old GE' (previous genetic engineering, see, for example, Liu et al., 2019; Gelvin et al., 2017; Forsbach et al., 2003; Jupe et al., 2019; Makarevitch et al., 2003; Windels et al., 2003; Rang et al., 2005). The example of Sanchez-Leon et al. (2018) is especially relevant when it comes to the discussion on unintended on-target effects such as also discussed in this Annex. In the case of this wheat, 35 out of 45 targeted alpha-gliadin genes were altered by CRISPR/Cas (SDN-1) to reduce gluten in food products. 1 This may appear to be a successful and precise application of the gene scissors, however, the changes lack sufficient predictability: there are many different types of insertions and/ or deletions which are specific to each of the targeted genes. In some cases, additional DNA was inserted into the target site. Consequently,

the large number of mutations required to make the wheat gluten-free is likely to require new approaches to correctly identify all gliadins and glutenins in the hexaploid genome of bread wheat, and also to identify the engineering strategy that introduced mutations of the correct nature and positions in each gene to prevent the accumulation of any peptide fragments associated with initiation of the inflammatory cascade (see EFSA, 2021). This case shows that, even where changes are 'successfully' introduced in the target genes, complex questions in regard to the safety of the plants need to be considered (see also Kawall, 2021): each and every targeted genetic site needs to undergo a detailed examination to investigate whether the alpha-gliadin proteins are still being produced, or if new proteins are produced unintentionally, or if any other unintended effects may occur. There is no doubt that such unintended variations of genetic changes caused by New GE (new genetic engineering techniques) can be associated with novel quality in hazards and risks, even if no off-target genetic changes are identified (see also e.g. Lalonde et al., 2017; Sharpe & Cooper, 2017). In addition, specific unintended on-target effects often include the integration of DNA from vector DNA derived from transformation processes, where, e.g. bacterial DNA is integrated (e.g. Li et al., 2015; Andersson et al. 2017, Zhang et al. 2018; Michno et al., 2020). Overall, the CRISPR/Cas9 system has been confirmed to have a high frequency of integration into the target site, resulting in large deletions at target sites (see, for example, Lee et al. 2019; Yang et al., 2022). Furthermore, the CRISPR/Cas machinery is particularly known for its potential to confuse target regions with specific off-target regions, and also for causing the unintended insertion of additional genes, the decoupling of genes and other specific genomic changes (of categories such as inversions, deletions or rearrangements), which are unlikely to emerge from spontaneous mutations or physical and chemical mutagenesis (see, for example, Biswas et al., 2020; Braatz et al., 2017; Höijer et al., 2022; Kawall et al., 2020). In some cases, unusual patterns of inheritance were also observed, as such escaping the Mendelian rules (Höijer et al., 2022; Yang, et al., 2022). Similarly to on-target genetic changes, off-target effects can also cause patterns of genetic change which go beyond what can be achieved with conventional breeding, resulting in specific and novel hazards and risks. Yang et al. (2022) gives an overview of irregular genetic changes and specific unintended effects caused by factors intrinsic to the CRISPR/Cas9 systems used in plants. These include off-target DNA cleavage, repetitive unit deletion, and indels of various sizes (see, for example, Zhang et al., 2014; Chakrarbarti et al., 2019; Manghwar et al., 2020; Molla and Yang, 2020; Kapusi et al., 2017; Kosicki et al., 2018). In this context, the dosage of CRISPR/Cas9 complexes expressed in cells can result in a significant increase of off-target mutation frequency (Ordon et al., 2017; Zhang et al., 2018).

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