APPROVED: 5 July 2022

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

European Food Safety Authority (EFSA)

Abstract/Summary

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 microorganisms obtained through synthetic biology. This draft scientific opinion was prepared by the EFSA Scientific Committee, supported by a Working Group on Synthetic Biology of Genetically Modified Micro-organisms. The draft opinion was endorsed by the EFSA Scientific Committee for public consultation on 17 November 2022. 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 11 different interested parties. EFSA and its Scientific Committee 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 Scientific Committee Plenary meeting on 5 July 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. In addition, BVL and Pollinis uploaded each an additional file with comments (See Appendix B) and Pollinis provided 2 reference documents.

The comments received were duly evaluated by the EFSA Scientific Committee Working Group on Synthetic Biology of Genetically Modified Micro-organisms. Wherever adequate 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 microorganisms 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 organisations that have submitted comments

Organisation Name[(a)]	**Country**
ANSES	France
AMFEP	Belgium
EFFCA - European Food and Feed Cultures Association	Belgium
Evonik Operations GmbH	Germany
EuropaBio - The European Association for Bioindustries	Belgium
Federal Office of Consumer protection and Food Safety (BVL), National Competent Authority	Germany
FEFANA asbl	Belgium
German Central Committee on Biological Safety (ZKBS)	Germany
JFDA	Jordan
POLLINIS	France
TestBiotech	Germany

(a): As specified by the commenter.

2. Comments received

Table 2: Comments and response from EFSA

comment ID	Organisation Name	Section Title	Comments	Responses from EFSA
1	Federal Office of Consumer protection and Food Safety (BVL), National Competent Authority	1. Introduction	Title: For the sake of consistency, the title should be changed by adding ? genetically modified ?: "Evaluation of existing guidelines for their adequacy for the food and feed risk assessment of genetically modified microorganisms obtained through synthetic biology" as it applied in SynBioP-Draft: ?Evaluation of existing guidelines for their adequacy for the food and feed risk assessment of genetically modified plants obtained through synthetic biology?	Title not changed to stay in line with the previous adopted Opinion on SynBioM MC and ERA.
2	ANSES	1. Introduction	No comment	na
3	Evonik Operations GmbH	1. Introduction	Evonik supports the industry input, which has been prepared and agreed to by AMFEP (the European Association of the Manufacturers and Formulators of Enzyme Products), EuropaBio (the European Association for Bioindustries) and FEFANA (the EU Association of Specialty Feed Ingredients and their Mixtures). In this response Evonik addresses the most critical aspects only.	na
4	FEFANA asbl	1. Introduction	This response was jointly prepared and is supported by AMFEP, FEFANA, and EuropaBio.	na
5	EuropaBio - The European Association	1. Introduction	This response was jointly prepared and is supported by AMFEP, FEFANA, and EuropaBio.	na

	for Bioindustries			
6	AMFEP	1. Introduction	This response was jointly prepared and is supported by AMFEP, FEFANA, and EuropaBio. ?	na
7	ANSES	1.1. Definitions for SynBio for the Terms of Reference	No comment	na
8	ANSES	1.2. Background and Terms of Reference as provided by the requestor	No comment	na
9	ANSES	1.3. Interpretation of the Terms of Reference	No comment	na
10	ANSES	1.4. Summary of the previous opinion on MC and ERA of SynBioM (EFSA Scientific Committee et al., 2020)	No comment	na
11	Evonik Operations GmbH	1.4. Summary of the previous opinion on MC and ERA of SynBioM (EFSA Scientific	Lines 125-126: We agree that a (new) EFSA guideline for microorganisms for deliberate release is needed. This guideline should be product-based rather that process-based to consider the fast development as well as the variety of different technologies resulting in similar changes.	Noted

		Committee et al., 2020)		
12	FEFANA asbl	1.4. Summary of the previous opinion on MC and ERA of SynBioM (EFSA Scientific Committee et al., 2020)	? Line 119: Please see our comments on ?extensively engineered? under the Glossary section below. ? Lines 125-126: We agree that a (new) EFSA guideline for microorganisms for deliberate release is needed. We encourage to ensure that this guideline becomes robust towards the fast development of technology anticipated. This goal can only be met by making the guidelines product-based rather than process-based as the variety of different technologies that will result in essentially similar changes will increase dramatically.	Noted idem
13	EuropaBio - The European Association for Bioindustries	1.4. Summary of the previous opinion on MC and ERA of SynBioM (EFSA Scientific Committee et al., 2020)	Line 119: Please see our comments on ?extensively engineered? under the Glossary section below. Lines 125-126: We agree that a (new) EFSA guideline for microorganisms for deliberate release is needed. We encourage to ensure that this guideline becomes robust towards the fast development of technology anticipated. This goal can only be met by making the guidelines product-based rather than process-based as the variety of different technologies that will result in essentially similar changes will increase dramatically.	idem
14	EFFCA - European Food and Feed Cultures Association	1.4. Summary of the previous opinion on MC and ERA of SynBioM (EFSA Scientific	EFFCA would like to support point raised by AMFEP, FEFANA, EuropaBio: ? Lines 125-126: We agree that a (new) EFSA guideline for microorganisms for deliberate release is needed. ?We encourage to ensure that this guideline becomes robust towards the fast development of technology ?anticipated. This goal can only be met	idem

		Committee et al., 2020)	by making the guidelines product-based rather than process-based ?as the variety of different technologies that will result in essentially similar changes will increase ?dramatically.	
15	AMFEP	1.4. Summary of the previous opinion on MC and ERA of SynBioM (EFSA Scientific Committee et al., 2020)	? Line 119: Please see our comments on ?extensively engineered? under the Glossary section below.? ? Lines 125-126: We agree that a (new) EFSA guideline for microorganisms for deliberate release is ?needed. We encourage to ensure that this guideline becomes robust towards the fast ?development of technology anticipated. This goal can only be met by making the guidelines ?product-based rather than process-based as the variety of different technologies that will result in ?essentially similar changes will increase dramatically. ?	idem
16	ANSES	2.1. Ad hoc expert Working Group and its methodology	No comment	na
17	ANSES	2.2. Consultations	No comment	na
18	Federal Office of Consumer protection and Food Safety (BVL), National Competent Authority	2.3. Existing guidances and guidelines checked in this Opinion	Table 2: This table illustrates how confusing and unclear the guidance situation is for GMM and other microbial products ? for the same GMM or other microbial product many guidances and/or even selected chapters of those are applicable. It has undoubtedly historical reasons, however the question arises, whether it would be more worthwhile to firstly update and to condense existing guidance materials for all microbial products prior to tackle adaptations regarding SynBioM in different guidance documents?	Noted. The comment is correct, but it needs to be considered that Microorganisms, GMMs/SynBioMs fall under different Regulatory frameworks according to their use. Moreover, this opinion does not include Guidance Development.

19	ANSES	2.3. Existing guidances and guidelines checked in this Opinion	No comment	na
20	Evonik Operations GmbH	2.3. Existing guidances and guidelines checked in this Opinion	It should be checked to which extent the documents referred to are relevant for microorganisms (e.g., document No 2). In addition, it should be checked whether the specified content and the referenced document match. For example, document No 28 does not provide information on how to assess potential risks resulting from horizontal gene transfer (HGT). This ?test guideline? describes an in vivo assay that detects chemicals that may induce gene mutations in somatic and germ cells.	Document 2 was removed from the Table 2 and used it in the text as a reference related to the assessment of horizontal gene transfer. Document 28 is indeed reference 2010b.
21	FEFANA asbl	2.3. Existing guidances and guidelines checked in this Opinion	It should be checked to which extent the documents referred to are relevant for microorganisms, see especially document No 2. In addition, it should be checked whether the specified content and the referenced document match. For example, document No 28 does not provide information on how to assess potential risks resulting from horizontal gene transfer (HGT). This Test Guideline describes an in vivo assay that detects chemicals that may induce gene mutations in somatic and germ cells. The correct reference should be OECD 2010b, according to the reference list.	idem

22	EuropaBio - The European Association for Bioindustries	2.3. Existing guidances and guidelines checked in this Opinion	It should be checked to which extent the documents referred to are relevant for microorganisms, see especially document No 2. In addition, it should be checked whether the specified content and the referenced document match. For example, document No 28 does not provide information on how to assess potential risks resulting from horizontal gene transfer (HGT). This Test Guideline describes an in vivo assay that detects chemicals that may induce gene mutations in somatic and germ cells. The correct reference should be OECD 2010b, according to the reference list.	idem
23	EFFCA - European Food and Feed Cultures Association	2.3. Existing guidances and guidelines checked in this Opinion	EFFCA would like to support point raised by AMFEP, FEFANA, EuropaBio:? It should be checked to which extent the documents referred to are relevant for microorganisms, see ?especially document No 2. In addition, it should be checked whether the specified content and the ?referenced document match. For example, document No 28 does not provide information on how to ?assess potential risks resulting from horizontal gene transfer (HGT). This Test Guideline describes an in ?vivo assay that detects chemicals that may induce gene mutations in somatic and germ cells. The ?correct reference should be OECD 2010b, according to the reference list.?	idem

24	AMFEP	2.3. Existing guidances and guidelines checked in this Opinion	It should be checked to which extent the documents referred to are relevant for microorganisms, see ?especially document No 2. In addition, it should be checked whether the specified content and the ?referenced document match. For example, document No 28 does not provide information on how to ?assess potential risks resulting from horizontal gene transfer (HGT). This Test Guideline describes an in ?vivo assay that detects chemicals that may induce gene mutations in somatic and germ cells. The ?correct reference should be OECD 2010b, according to the reference list.?	Idem
25	Federal Office of Consumer protection and Food Safety (BVL), National Competent Authority	2.4. Categories of products, use applications and legal frameworks covered in this Opinion	Figure 1: Does this list base on horizon scanning/systematic literature search? Insertion of a reference might be helpful	Added "as expected by the WG in the near future ".
26	ANSES	2.4. Categories of products, use applications and legal frameworks covered in this Opinion	Although the context is implied in the description of the 4 categories, a column may be missing from this table which more precisely indicates the context of use of the various genetically modified microbial strains (fermenter, human digestive tract , ground, etc.). This information is necessary to fully understand the classification into different categories and prioritize the risks. Indeed, an MGM that passes through the digestive tract to subsequently end up in the environment requires	The routes of exposure are considered sufficiently clear when the use application is clarified for the product, hence this info is already covered in the text where examples are mentioned.

			much more sustained attention than an MGM confined in a fermenter and subsequently destroyed.	
27	Evonik Operations GmbH	2.4. Categories of products, use applications and legal frameworks covered in this Opinion	This is a very critical point for us. In this section EFSA expresses the view that presence of recombinant DNA is a trigger for the applicability of Regulation (EC) No 1829/2003 on GM food and feed. This view, recently postulated by some stakeholders, has been contested by a recent legal opinion by Prof. Dederer from the University of Passau in Germany. According to this, only the absence of the GMM, and not DNA, is a legal requirement for being exempted from Regulation (EC) No 1829/2003 (https://stoffr.lexxion.eu/article/STOFFR/2021/3/6). Regulatory classification is in the remit of the Commission and should not be covered here. Therefore, we propose to: ? Delete the two sentences from line 177 to line 181; ? Delete the sentence from line 184 to line 186; and ? Delete the reference to Regulation (EC) No 1829/2003 in Figure 1 for products of Category 3.	References to legal frameworks have been deleted.

28	FEFANA asbl	2.4. Categories of products, use applications and legal frameworks covered in this Opinion	EFSA in this section expresses the view that presence of recombinant DNA is a trigger for the applicability of Regulation (EC) No 1829/2003. This view, recently postulated by some stakeholders, has been contested by a recent legal opinion by Prof. Dederer from the University of Passau in Germany. According to Professor Dederer?s assessment, only the absence of the GMM, and not DNA, is a legal requirement for being exempted from Regulation (EC) No 1829/2003 (https://stoffr.lexxion.eu/article/STOFFR/2021/3/6). However, we appreciate that EFSA states, in alignment with Professor Dederer, in lines 181-184 that GMM products in categories 1 and 2 are produced with GMM, not from GMM. This excludes them from the scope of Regulation (EC) No 1829/2003. In addition, this Opinion should only cover aspects relevant to the safety assessment which are in the remit of EFSA. Regulatory classification is in the remit of the Commission and should not be covered here. Therefore, we propose to: ? Delete the two sentences from line 177 to line 181 ? Delete the sentence from line 184 to line 186 ? Delete the reference to Regulation (EC) No 1829/2003 in Figure 1 for products of Category 3	idem

29	EuropaBio - The European Association for Bioindustries	2.4. Categories of products, use applications and legal frameworks covered in this Opinion	EFSA in this section expresses the view that presence of recombinant DNA is a trigger for the applicability of Regulation (EC) No 1829/2003. This view, recently postulated by some stakeholders, has been contested by a recent legal opinion by Prof. Dederer from the University of Passau in Germany. According to Professor Dederer?s assessment, only the absence of the GMM, and not DNA, is a legal requirement for being exempted from Regulation (EC) No 1829/2003 (https://stoffr.lexxion.eu/article/STOFFR/2021/3/6). However, we appreciate that EFSA states, in alignment with Professor Dederer, in lines 181-184 that GMM products in categories 1 and 2 are produced with GMM, not from GMM. This excludes them from the scope of Regulation (EC) No 1829/2003. In addition, this Opinion should only cover aspects relevant to the safety assessment which are in the remit of EFSA. Regulatory classification is in the remit of the Commission and should not be covered here. Therefore, we would suggest to: ? Delete the two sentences from line 177 to line 181 ? Delete the sentence from line 184 to line 186 ? Delete the reference to Regulation (EC) No 1829/2003 in Figure 1 for products of Category 3	idem

30	EFFCA - European Food and Feed Cultures Association	2.4. Categories of products, use applications and legal frameworks covered in this Opinion	EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? EFSA in this section expresses the view that presence of recombinant DNA is a trigger for the applicability ?of Regulation (EC) No 1829/2003. This view, recently postulated by some stakeholders, has been ?contested by a recent legal opinion by Prof. Dederer from the University of Passau in Germany. According ?to Professor Dederer?s assessment, only the absence of the GMM, and not DNA, is a legal requirement for ?being exempted from Regulation (EC) No 1829/2003 (https://stoffr.lexxion.eu/article/STOFFR/2021/3/6). ? However, we appreciate that EFSA states, in alignment with Professor Dederer, in lines 181-184 that ?GMM products od categories 1 and 2 are produced with GMM, not from GMM. This excludes them from ?the scope of Regulation (EC) No 1829/2003.? In addition, this Opinion should only cover aspects relevant to the safety assessment which are in the ?remit of EFSA. Regulatory classification is in the remit of the Commission and should not be covered here. ?Therefore, we propose to:? ? Delete the two sentences from line 177 to line 181? ? Delete the sentence from line 184 to line 186? ? Delete the reference to Regulation (EC) No 1829/2003 in Figure 1 for products of Category 3?	idem

31	AMFEP	2.4. Categories of products, use applications and legal frameworks covered in this Opinion	CRITICAL: EFSA in this section expresses the view that presence of recombinant DNA is a trigger for the ?applicability of Regulation (EC) No 1829/2003. This view, recently postulated by some stakeholders, has ?been contested by a recent legal opinion by Prof. Dederer from the University of Passau in Germany. ?According to Professor Dederer?s assessment, only the absence of the GMM, and not DNA, is a legal ?requirement for being exempted from Regulation (EC) No 1829/2003 ??(https://stoffr.lexxion.eu/article/STOFFR/2021/3/6). ? However, we appreciate that EFSA states, in alignment with Professor Dederer, in lines 181-184 that ?GMM products in categories 1 and 2 are produced with GMM, not from GMM. This excludes them from ?the scope of Regulation (EC) No 1829/2003.? In addition, this Opinion should only cover aspects relevant to the safety assessment which are in the ?remit of EFSA. Regulatory classification is in the remit of the Commission and should not be covered here. ?Therefore, we would suggest to:? ? Delete the two sentences from line 177 to line 181? ? Delete the sentence from line 184 to line 186? ? Delete the reference to Regulation (EC) No 1829/2003 in Figure 1 for products of Category 3?	idem

32	Federal Office of Consumer protection and Food Safety (BVL), National Competent Authority	2.5. Selection of case studies	Table 3: As the authors rightly state there is no commonly agreed definition for Synthetic Biology. It is thus difficult to identify cases for the case studies that differ sufficiently from classical genetic engineering. The cases 1 to 3 can be considered classical examples of synthetic biology. Other cases, for example case 4, in which a nucleotide sequence from the same species is introduced into A. oryzae, or case 7, which is a classical application of a CRISPR interference system to block gene expression, are standard genetic engineering. These classic cases could have been left out to better concentrate on the cases where novelty is greater. However, the document rightly analyses that these cases are already covered by existing regulation or if adaptations are needed these are not due to the genetically engineered nature of the products (e. g. phages).	All cases presented are examples of genetic modifications, organisms or desired traits which are not yet notified to EFSA. These cases represent a different range of genetic modification which can be expected from the near to the wider future. Techniques and SynBio approaches used in the cases were better clarified in the text.
33	ANSES	2.5. Selection of case studies	Case 3 : MG In what context are these MGMs used (production of enzyme? release of the MGM into the environment?) Case 4 : It is not clear here if the modified strain is intended to be implanted in the digestive tract of humans or animals.	Different use applications are possible for Case 3 (see column 2). Case 3 presents the actual development in the field of xenobiology. The article was used to present at the different hypothetical synthetic microorganisms (for the different categories) which could be developed in the future based on this technology. In case 4 the modified strain is not viable and would therefore not be able to proliferate in the digestive tract of humans or animals.
34	Evonik Operations GmbH	2.5. Selection of case studies	Line 231: The section 4.3 referred to in this line does not exist and should be replaced by section 4.2. Please check also line 821 as section 4.3 is also referred to there.	Changes were made.

35	FEFANA asbl	2.5. Selection of case studies	? Line 231: The section 4.3 referred to in this line does not exist and should be replaced by section 4.2. Please check also line 821 as section 4.3 is also referred to there. The case studies selected by EFSA support the opening statement in this section that the border between ?genetic engineering? and ?synthetic biology? cannot be clearly defined, which raises the question whether at all, or if so, for which products/applications of synthetic biology, additional guidance might be needed. Clear examples of ?genetic engineering?, which were done long before the term synthetic biology was even coined, include: ? Case study 4: Combination of classical mutagenesis and overexpression of creA. ? Case study 8: Metabolic engineering in a classical way. In addition, case study 1 that seemingly could be a minimal cell approach is a traditional strain engineering approach with multiple serial deletions of DNA elements in B. subtilis A168. Much of this work was done about 20 years ago.	Idem as above comment 32 and 34. All cases presented are examples of genetic modifications, organisms or desired traits which are not yet notified to EFSA. These cases represent a different range of genetic modification which can be expected from the near to the wider future. Techniques and SynBio approaches used in the cases were better clarified in the text.

36	EuropaBio - The European Association for Bioindustries	2.5. Selection of case studies	Line 231: The section 4.3 referred to in this line does not exist and should be replaced by section 4.2. Please check also line 821 as section 4.3 is also referred to there. The case studies selected by EFSA support the opening statement in this section that the border between ?genetic engineering? and ?synthetic biology? cannot be clearly defined, which raises the question whether at all, or if so, for which products/applications of synthetic biology, additional guidance might be needed. Clear examples of ?genetic engineering?, which were done long before the term synthetic biology was even coined, include: Case study 4: Combination of classical mutagenesis and overexpression of creA. Case study 8: Metabolic engineering in a classical way. In addition, case study 1 that seemingly could be a minimal cell approach is a traditional strain engineering approach with multiple serial deletions of DNA elements in B. subtilis A168. Much of this work was done about 20 years ago.	idem
37	German Central Committee on Biological Safety (ZKBS)	2.5. Selection of case studies	The paper states that there is ?no distinct borderline between the microorganisms obtained using existing genetic modification techniques and those derived from synthetic biology? and thus considers 13 case studies that range from classical genetically modified to less familiar SynBio microorganisms. The authors themselves consider the cases 1 to 3 as less familiar. It would have been better to concentrate on these three cases to better stick to the subject, i.e. the evaluation of SynBio microorganisms in food and feed. Further, the case studies show that most aspects of the	idem

			three least familiar cases 1 to 3 can be assessed with the already existing guidances as these organisms are considered GMOs.	
38	EFFCA - European Food and Feed Cultures Association	2.5. Selection of case studies	EFFCA would like to support point raised by AMFEP, FEFANA, EuropaBio:? ? Line 231: The section 4.3 referred to in this line does not exist and should be replaced by section ??4.2. Please check also line 821 as section 4.3 is also referred to there.? The case studies selected by EFSA support the opening statement in this section that the border between ???genetic engineering? and ?synthetic biology? cannot be clearly defined, which raises the question ?whether at all, or if so, for which products/applications of synthetic biology, additional guidance might be ?needed. Clear examples of ?genetic engineering?, which were done long before the term synthetic biology ?was even coined, include:? Case study 4: Combination of classical mutagenesis and overexpression of creA.? Case study 8: Metabolic engineering in a classical way.? In addition, case study 1 that seemingly could be a minimal cell approach is a traditional strain ?engineering approach with multiple serial deletions of DNA elements in B. subtilis A168. Much of this ?work was done about 20 years ago.?	idem

39	AMFEP	2.5. Selection of case studies	? Line 231: The section 4.3 referred to in this line does not exist and should be replaced by section ??4.2. Please check also line 821 as section 4.3 is also referred to there.? The case studies selected by EFSA support the opening statement in this section that the border between ???genetic engineering? and ?synthetic biology? cannot be clearly defined, which raises the question ?whether at all, or if so, for which products/applications of synthetic biology, additional guidance might be ?needed. Clear examples of ?genetic engineering?, which were done long before the term synthetic biology ?was even coined, include:? Case study 4: Combination of classical mutagenesis and overexpression of creA.? Case study 8: Metabolic engineering in a classical way.? In addition, case study 1 that seemingly could be a minimal cell approach is a traditional strain ?engineering approach with multiple serial deletions of DNA elements in B. subtilis A168. Much of this ?work was done about 20 years ago.?	idem

40	Testbiotech	2.5. Selection of case studies	What is missing in the cases studies are microorganisms which are meant to produce biologically active molecules, such as non-coding RNAs (ncRNAs), that influence the characteristics of food-producing animals (such as bees or livestock) or plants (for example, via micorrhiza or endobionts), which may enter the food production chain directly or indirectly, intentionally or unintentionally. The concept of paratransgenesis should also be mentioned in this context. These issues go beyond what is indicated for plant protection products, and the need for updated guidance is likely to not only concern exposure. In addition, the application of SynbBio microorganisms, which may deliver health effects in humans via ncRNA when ingested directly, should also be integrated in the case studies. Such applications are also of relevance in the discussion regarding effects on the microbiome.	Two new case studies were introduced to address this comment.

41	POLLINIS	2.5. Selection of case studies	EFSA states: ?There is no distinct borderline between the microorganisms obtained using existing genetic modification techniques and those derived from synthetic biology? [1]. We would suggest to add two issues to be included into the guidelines: 1. Understanding that this is not ERA, still, we think that there needs to special attention on a focus on more larger scales and long-term studies to understand the effects of applications of synthetic biology on microorganisms. Also understanding better concepts of invasion ecology: introduction of new organisms that many come from other continents. One of the lessons learned from invasion ecology is an increased transfer (intended and unintended) between organisms. Proponents argue that if there a large number of introductions, as in microorganisms, there will be a definitive impact on the environment and could have disastrous effects on the environment [2]. 2. We also think it is a good idea that case studies include microorganisms that are used for organisms that influence food-affected animals, especially the honey bee (see below for comments on gut microbiome ? 3.8). The idea of paratransgenesis [3-7] should be included in these guidelines as these issues go beyond just the microorganism, but the organism and overall environment, including human health (e.g. plant protection products, pest control) [4].	1. ERA was addressed in opinion 1 (https://www.efsa.europa.eu/en/efsajournal/pub/6263) and this opinion includes checking the interactions with the receiving environment. 2. Idem as comment 40
42	ANSES	2.Data and Methodologies	No comment	na
43	ANSES	3. Assessment	No comment	na

44	ANSES	3.1. General outline of risk assessment for genetically modified microorganisms	No comment	na
45	ANSES	3.10. Nutritional assessment	No comment	na
46	German Central Committee on Biological Safety (ZKBS)	3.10. Nutritional assessment	The Guidance on Novel Food Applications may be applicable to xenobionts containing XNA and/or producing xenoproteins, if the term ?DNA? is exchanged with ?nucleic acids?.	Noted. The presence of XNA and/or xenoproteins would require the development of adapted guidance which could be based on general principles already formulated in the now existing guidances
47	Testbiotech	3.10. Nutritional assessment	New guidelines are needed on how to generally include the potential impacts and interactions of accumulated and combinatorial effects caused by the presence of more than one SynBioM in a joint environment, by taking into account specific scenarios. New guidance is needed on how to detect, identify, monitor and control the unintended presence of SynBioM and their DNA or XNA.	Noted. Accumulated and combinatorial effects: This is outside of the scope of the Terms of Reference of this opinion and not specifically related to Synthetic Biology but to all risk assessments. Case-by-case assessment per product is currently foreseen in legal frameworks, which are implemented by EFSA RAs. ERA for synthetic biology was addressed in a first opinion (https://www.efsa.europa.eu/en/efsajournal/pub/6263) and includes interactions with the environment. Detection, identification as well as PMM has been addressed in that first opinion and recommendations were formulated where relevant.

48	Federal Office of Consumer protection and Food Safety (BVL), National Competent Authority	3.11. Exposure assessment	Table 13, case 13: It must be stressed here that the need for updates is not connected to the SynBio properties	Noted. Indeed, in the conclusions of this opinion, (also in the conclusion of the exposure assessment), it has been specified if the need for updates is recommended for non-GM, GM and SynBioM where relevant.
49	ANSES	3.11. Exposure assessment	No comment	na
50	Evonik Operations GmbH	3.11. Exposure assessment	Line 821: The section 4.3 referred to does not exist and should be replaced by section 4.2.	Change made

51	FEFANA asbl	3.11. Exposure assessment	? Lines 800 ? 814: It is argued that secondary routes for exposure need to be considered. This is the case both for non-SynBio and SynBio organisms. Particularly for the example (case 13) the assessment needed only relates to the properties of the Pseudomonas strain, not the way it was constructed. A strain like this could also be constructed using non SynBio techniques. This is reflected also on lines 842-844. ? Line 821: The section 4.3 referred to does not exist and should be replaced by section 4.2. ? Lines 842-844: Oral exposure assessment, as indicated here, is a very complex topic. Likely this would be based on the investigation of potential impacts on the gut microbiome. As already mentioned in the comments to section 3.8, a scientifically founded definition of a healthy gut microbiome must be established before any exposure assessment can be explored. Moreover, the risk posed by such organisms is very low. Exposure is also not comparable to microbes used for intentional ingestion in food/feed products. Also, EFSA intends to include non-GM and GMM microorganisms and their metabolites into this assessment. This might not be within the scope of this evaluation and accordingly, ?non-GM? and ?GMM? should be deleted from the sentence in line 842. Moreover, exposure assessment should be required only in case of the presence of genes of concern or XNA in the microorganism.	Secondary routed for exposure: EFSA notes this comment and decided to keep the exposure assessment in a broad context, highlighting the need for guidance update for non-GM, GM and SynBioM. EFSA considers exposure assessment an essential element of the risk assessment. Noted: text changed for various points where relevant and as addressed in comment 96,98.

52	EuropaBio - The European Association for Bioindustries	3.11. Exposure assessment	Lines 800 ? 814: It is argued that secondary routes for exposure need to be considered. This is the case both for non-SynBio and SynBio organisms. Particularly for the example (case 13) the assessment needed only relates to the properties of the Pseudomonas strain, not the way it was constructed. A strain like this could also be constructed using non SynBio techniques. This is reflected also on lines 842-844. Line 821: The section 4.3 referred to does not exist and should be replaced by section 4.2. Lines 842-844: Oral exposure assessment, as indicated here, is a very complex topic. Likely this would be based on the investigation of potential impacts on the gut microbiome. As already mentioned in the comments to section 3.8, a scientifically founded definition of a healthy gut microbiome must be established before any exposure assessment can be explored. Moreover, the risk posed by such organisms is very low. Exposure is also not comparable to microbes used for intentional ingestion in food/feed products. Also, EFSA intends to include non-GM and GMM microorganisms and their metabolites into this assessment. This might not be within the scope of this evaluation and accordingly, ?non-GM? and ?GMM? should be deleted from the sentence in line 842. Moreover, exposure assessment should be required only in case of the presence of genes of concern or XNA in the microorganism.	idem
53	EFFCA - European Food and	3.11. Exposure assessment	EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? ? Lines 800 ? 814: It is argued that secondary routes for	idem

	Feed Cultures Association		exposure need to be considered. This is the ?case both for non-SynBio and SynBio organisms. Particularly for the example (case 13) the ?assessment needed only relates to the properties of the Pseudomonas strain, not the way it was ?constructed. A strain like this could also be constructed using non SynBio techniques. This is ?reflected also on lines 842-844.? ? Line 821: The section 4.3 referred to does not exist and should be replaced by section 4.2. ? ? Lines 842-844: Oral exposure assessment, as indicated here, is a very complex topic. Likely this ?would be based on the investigation of potential impacts on the gut microbiome. As already ?mentioned in the comments to section 3.8, a scientifically founded definition of a healthy gut ?microbiome must be established before any exposure assessment can be explored. Moreover, the risk posed by such organisms is very low for and also exposure is not comparable ?to microbes used for intentional ingestion in food/feed products. The suggestion made by EFSA ?seems to be more driven by scientific interest than by actual safety concern. Also, EFSA intends to include non-GM and GMM microorganisms and their metabolites into this ?assessment. This might not be within the scope of this evaluation and accordingly, ?non-GM? and ???GMM? should be deleted from the sentence in line 842. Moreover, exposure assessment should ?be required only in case of the presence of genes of concern or XNA in the microorganism.?	

54	AMFEP	3.11. Exposure assessment	? Lines 800 ? 814: It is argued that secondary routes for exposure need to be considered. This is the ?case both for non-SynBio and SynBio organisms. Particularly for the example (case 13) the ?assessment needed only relates to the properties of the Pseudomonas strain, not the way it was ?constructed. A strain like this could also be constructed using non SynBio techniques. This is ?reflected also on lines 842-844.? ? Line 821: The section 4.3 referred to does not exist and should be replaced by section 4.2. ? ? Lines 842-844: Oral exposure assessment, as indicated here, is a very complex topic. Likely this ?would be based on the investigation of potential impacts on the gut microbiome. As already ?mentioned in the comments to section 3.8, a scientifically founded definition of a healthy gut ?microbiome must be established before any exposure assessment can be explored. Moreover, the ?risk posed by such organisms is very low. Exposure is also not comparable to microbes used for ?intentional ingestion in food/feed products. Also, EFSA intends to include non-GM and GMM ?microorganisms and their metabolites into this assessment. This might not be within the scope of ?this evaluation and accordingly, ?non-GM? and ?GMM? should be deleted from the sentence in ?line 842. Moreover, exposure assessment should be required only in case of the presence of ?genes of concern or XNA in the microorganism?	idem

55	Testbiotech	3.11. Exposure assessment	New guidelines are needed on how to generally include the potential impacts and interactions of accumulated and combinatorial effects caused by the presence of more than one SynBioM in a joint environment, by taking into account specific scenarios. New guidance is needed on how to detect, identify, monitor and control the unintended presence of SynBioM and their DNA or XNA.	idem as comment 47.
56	ANSES	3.12. Post-market monitoring	No comment	na
57	Evonik Operations GmbH	3.12. Post-market monitoring	Lines 850, 853, and 880: ?Derived from? is not a term used in the EU GMO regulatory framework. ?Derived from? should be replaced by ?produced from? throughout the document. Lines 882-883: Such ?fit-for-purpose approaches? to monitor for potential adverse effects of microorganisms should be science-based and proportionate to the potential risk. The scientific basis should be internationally recognized publications that also survive review by experts in the field and not ?only? by the reviewers of the corresponding journal.	Text adjusted. Updating guidance for general requirements of post market monitoring is not within the ToR of this opinion.

58	FEFANA asbl	3.12. Post-market monitoring	? Lines 850, 853, and 880: ?Derived from? is not a term used in the EU GMO regulatory framework. Therefore: ?derived from? should be replaced in lines 850, 853 and 880 by ?produced from?. ? Lines 882-883: Such ?fit-for-purpose approaches? to monitor for potential adverse effects of microorganisms should be science-based and proportionate to the potential risk. The scientific basis should be internationally recognized publications that also survive review by experts in the field. ? While current guidance is deemed adequate, the desire to include fit-for-purpose approaches to monitor for potential adverse effects of microorganisms (non-GM, GM and SynBioM) could substantially increase the burden for organisms which have been deemed safe already or are QPS strains. Such additional monitoring should carefully consider the product-associated risk (for non-GM, GM and SynBioM) before requiring such actions.	idem. Post market monitoring should be required only in specific cases as is mentioned in the text.
59	EuropaBio - The European Association for Bioindustries	3.12. Post-market monitoring	Lines 850, 853, and 880: ?Derived from? is not a term used in the EU GMO regulatory framework. Therefore: ?derived from? should be replaced in lines 850, 853 and 880 by ?produced from?. Lines 882-883: Such ?fit-for-purpose approaches? to monitor for potential adverse effects of microorganisms should be science-based and proportionate to the potential risk. The scientific basis should be internationally recognized publications that also survive review by experts in the field. While current guidance is deemed adequate, the desire to include fit-for-purpose	idem

			approaches to monitor for potential adverse effects of microorganisms (non-GM, GM and SynBioM) could substantially increase the burden for organisms which have been deemed safe already or are QPS strains. Such additional monitoring should carefully consider the product-associated risk (for non-GM, GM and SynBioM) before requiring such actions.	
60	EFFCA - European Food and Feed Cultures Association	3.12. Post-market monitoring	EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? ? Lines 850, 853, and 880: ?Derived from? is not a term used in the EU GMO regulatory ?framework. Therefore: ?derived from? should be replaced in lines 850, 853 and 880 by ???produced from?. ? ? Lines 882-883: Such ?fit-for-purpose approaches? to monitor for potential adverse effects of ?microorganisms should be science-based and proportionate to the potential risk. The scientific ?basis should be internationally recognized publications that also survive review by experts in the ?field and not ?only? by the reviewers of the corresponding journal.? ? While current guidance is deemed adequate, the desire to include fit-for-purpose approaches to ?monitor for potential adverse effects of microorganisms (non-GM, GM and SynBioM) could ?substantially increase the burden for organisms which have been deemed safe already or are QPS ?strains. Such additional monitoring should carefully consider the product-associated risk (for non-?GM, GM and SynBioM) before requiring such actions?.	idem

61	AMFEP	3.12. Post-market monitoring	? Lines 850, 853, and 880: ?Derived from? is not a term used in the EU GMO regulatory ?framework. Therefore: ?derived from? should be replaced in lines 850, 853 and 880 by ???produced from?. ? ? Lines 882-883: Such ?fit-for-purpose approaches? to monitor for potential adverse effects of ?microorganisms should be science-based and proportionate to the potential risk. The scientific ?basis should be internationally recognized publications that also survive review by experts in the ?field.? ? While current guidance is deemed adequate, the desire to include fit-for-purpose approaches to ?monitor for potential adverse effects of microorganisms (non-GM, GM and SynBioM) could ?substantially increase the burden for organisms which have been deemed safe already or are QPS ?strains. Such additional monitoring should carefully consider the product-associated risk (for non-?GM, GM and SynBioM) before requiring such actions.?	idem
62	Testbiotech	3.12. Post-market monitoring	New guidelines are needed on how to generally include the potential impacts and interactions of accumulated and combinatorial effects caused by the presence of more than one SynBioM in a joint environment, by taking into account specific scenarios. New guidance is needed on how to detect, identify, monitor and control the unintended presence of SynBioM and their DNA or XNA.	idem as above comment 47

63	ANSES	3.2. Assessment of the ?Categorisation of the GMMs and their products for risk assessment purposes? of the EFSA GMO Panel Guidance (2011)?	No comment	na
64	FEFANA asbl	3.2. Assessment of the ?Categorisation of the GMMs and their products for risk assessment purposes? of the EFSA GMO Panel Guidance (2011)?	? Line 290: We support the suggestion made in Table 4 and in line 290 to merge the categories 1 and 2 defined in the EFSA GMO Panel guidance from 2011. This will facilitate future guidance and assessment of applications. ? Lines 288 ? 289: To be in line with the conclusions made in Table 4 for cases 1 and 3, we suggest clarifying in lines 288-289 that categories 1 and 2 are not only not distinguished in practice, but they also cannot be distinguished.	Noted. The original Cat 1 and Cat 2 distinction is still possible based on the purity of the product (but not done in practice)
65	EuropaBio - The European Association for Bioindustries	3.2. Assessment of the ?Categorisation of the GMMs and their products for risk assessment purposes? of the EFSA GMO Panel Guidance (2011)?	Lines 288 ? 289: To be in line with the conclusions made in Table 4 for cases 1 and 3, we suggest clarifying in lines 288-289 that categories 1 and 2 are not only not distinguished in practice, but they also cannot be distinguished. Line 290: We support the suggestion made in Table 4 and in line 290 to merge the categories 1 and 2 defined in the EFSA GMO Panel guidance from 2011. This will facilitate future guidance and assessment of applications.	idem

66	AMFEP	3.2. Assessment of the ?Categorisation of the GMMs and their products for risk assessment purposes? of the EFSA GMO Panel Guidance (2011)?	? Line 290: We support the suggestion made in Table 4 and in line 290 to merge the categories 1 ?and 2 defined in the EFSA GMO Panel guidance from 2011. This will facilitate future guidance and ?assessment of applications. ? ? Lines 288 ? 289: To be in line with the conclusions made in Table 4 for cases 1 and 3, we suggest ?clarifying in lines 288-289 that categories 1 and 2 are not only not distinguished in practice, but ?they also cannot be distinguished.?	idem
67	Federal Office of Consumer protection and Food Safety (BVL), National Competent Authority	3.3. Microbial characterisation including QPS evaluation	Table 5: The table legend is misleading and not self-explanatory, especially the last sentence. It is difficult to compare the table with the table 3 (not 2!) to check which guidances may be considered besides GMM 2011. It could be beneficial and easier for reading to insert an additional column indicating which additional guidance (if any) or selected chapter were considered during the assessment for each example. Table 5, case 1: Are there studies or examples on QPS-Strains, which underpin this assumption? Generally, QPS strains such as B. subtilis have no or only very few pathogenicity-related genes. It is thus highly unlikely that they become pathogenic when part of the genome is deleted. A large genomic deletion will also lead to a replication defect if the strain is not cultured under specific conditions such as a complemented culture medium. Finally, bacteria are usually recognised by the host´s immune system through several antigens including antigens in their cell wall. It is unlikely that all antigens present in B. subtilis are deleted	Table 2 was changed to Table 3 in the legend of Table 5 and all other tables of the assessment chapter. Genome minimisation and possible risk has been revisited in the opinion with relevant references. Indeed, for case 4 the QPS approach is not applicable for this organism group, now mentioned in the table column 3.

			in the genome-reduced strain. Table 5, case 4: It must be stressed here, that the QPS approach is not applicable in this case due to the absence of Aspergillus oryzae in the QPS list, not due to its SynBio properties.	
68	ANSES	3.3. Microbial characterisation including QPS evaluation	It might be useful to find an easier presentation as understanding Table 5 needs going back and forth to Table 4. Does this include the concern on the possible loss of antigens that could render the SynBioM invisible to the immune system, therefore altering the safety status of the organism. How can this be predicted with confidence ? Same question for case studies 7-9 and 11-13, all in category 4.	No changes made in the presentation of the Tables in order to avoid repetition. See answer above for comment 67.
69	FEFANA asbl	3.3. Microbial characterisation including QPS evaluation	? Table 5, Case 1: ?Minimisation may lead to new features of concern, e.g. to the loss of antigens that could render the SynBioM invisible to the immune system, therefore altering the safety status of the organism.? This seems to be an unsubstantiated hypothesis rather than a validated risk for which a substantial scientific rationale and basis is available. If it is the former, the text should be deleted. If it is the latter, a proper reference should be provided, supporting this potential risk. ? The lowest taxonomic unit to which QPS status is assigned currently is the species. Considering the merits of the QPS approach for both EFSA and applicants, we recommend expanding the QPS concept also to the sub-species level (e.g. E. coli K12) and to lineages of strains with scientifically demonstrated ?intrinsic? safety.	idem as for comment 67. EFSA notes the suggestion made for extending the QPS approach to certain lineages (which is outside the scope of this opinion).

70	EuropaBio - The European Association for Bioindustries	3.3. Microbial characterisation including QPS evaluation	Table 5, Case 1: ?Minimisation may lead to new features of concern, e.g. to the loss of antigens that could render the SynBioM invisible to the immune system, therefore altering the safety status of the organism.? This seems to be an unsubstantiated hypothesis rather than a validated risk for which a substantial scientific rationale and basis is available. If it is the former, the text should be deleted. If it is the latter, a proper reference should be provided, supporting this potential risk. The lowest taxonomic unit to which QPS status is assigned currently is the species. Considering the merits of the QPS approach for both EFSA and applicants, we recommend expanding the QPS concept also to the sub-species level (e.g. E. coli K12) and to lineages of strains with scientifically demonstrated ?intrinsic? safety.	idem.
71	EFFCA - European Food and Feed Cultures Association	3.3. Microbial characterisation including QPS evaluation	EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? ? Table 5, Case 1: ?Minimisation may lead to new features of concern, e.g. to the loss of antigens ?that could render the SynBioM invisible to the immune system, therefore altering the safety ?status of the organism.? This seems to be an unsubstantiated hypothesis rather than a validated ?risk for which a substantial scientific rationale and basis is available. If it is the former, the text ?should be deleted. If it is the latter, a proper reference should be provided, supporting this ?potential risk.? ? The lowest taxonomic unit to which QPS status is assigned currently is the species. Considering ?the merits of the QPS	idem

			approach for both EFSA and applicants, we recommend expanding the QPS ?concept also to the sub-species level (e.g. E. coli K12) and to lineages of strains with scientifically ?demonstrated ?intrinsic? safety.?	
72	AMFEP	3.3. Microbial characterisation including QPS evaluation	? Table 5, Case 1: ?Minimisation may lead to new features of concern, e.g. to the loss of antigens ?that could render the SynBioM invisible to the immune system, therefore altering the safety ?status of the organism.? This seems to be an unsubstantiated hypothesis rather than a validated ?risk for which a substantial scientific rationale and basis is available. If it is the former, the text ?should be deleted. If it is the latter, a proper reference should be provided, supporting this ?potential risk.? ? The lowest taxonomic unit to which QPS status is assigned currently is the species. Considering ?the merits of the QPS approach for both EFSA and applicants, we recommend expanding the QPS ?concept also to the sub-species level (e.g. E. coli K12) and to lineages of strains with scientifically ?demonstrated ?intrinsic? safety.?	idem
73	Testbiotech	3.3. Microbial characterisation including QPS evaluation	One weakness of the QPS concept are potential interactions between the newly introduced SynBio traits and the characteristics of the wild type microorganisms. Guidance will be needed on how to assess these potentially complex interactions within the cells and between the organisms. The guidelines should include the evaluation of the QPS concept in regard to ? unintended interactions within the cells; ? new phenotypical characteristics of the cell populations within one	The QPS assessment takes into account all the potential negative effects of a microbial species (taxonomical unit), including possible gene transfer and interactions (see the latest QPS opinion https://www.efsa.europa.eu/en/efsajournal/pub/5966). See reply to comment 47 for possible combinatorial effects.

			species (such as growth of bacteria); ? potential interactions, accumulated and combinatorial effects between organisms caused by the (intended or unintended) presence of more than one SynBioM in a specific environment.	
74	Federal Office of Consumer protection and Food Safety (BVL), National Competent Authority	3.4. Information relating to the product, information relating to the production process and information relating to the product preparation process (several guidances)	Table 6, case 5: The reasoning is not clear here. According to the GMM 2011, the requirements for this section are to provide detailed description of fermentation processes, inactivation methods, and methods for the proof of absence of viable cells. These requirements are not limited to certain trophic metabolic phenotypes. Even though the auxotrophic fermentation is innovative, the requirement for the production process are still valid and this guidance should be considered adequate. It must be stressed here that the need for updates is not connected to the Synbio properties Table 6 case 4: What is about the enzyme guidance (Table 3), is it also adequate? Table 6 Case 6, 10, and 11: It must be stressed here that the need for updates is not connected to the SynBio properties Line 376: It is not clear to which footnote the text is referring here	Text adjusted into adequate for case 5 and for case 4. In the conclusions of the Opinion a differentiation is made when the conclusion and the need for updates is applying to SynBioM or when applying to broader microorganism types (non-GM, GMM or SynBioM). Line 376: this footnote has been removed as section 2.4 is redrafted.

75	ANSES	3.4. Information relating to the product, information relating to the production process and information relating to the product preparation process (several guidances)	Not clear what is inadequate in the guidance for this case study 5 ? Case 10 : EFSA's opinion on the use of Listex to limit the development of Listeria monocytogenes is rather unfavourable. If the legislation is not very clear for bacteriophages that are not genetically modified, how can we design a guide even in some time for those that are genetically modified ? Not only the transduction of virulence genes but in general any transduction. Transducer phages can subsequently transfer bacterial genes to other strains; Admittedly, this is a phenomenon that occurs naturally in ecosystems, but it can be significantly accelerated to the extent that very large quantities of phage particles are produced.	case 5: text adjusted. Case 10: EFSA takes note of this comment. The conclusion mentions the need for guidance for non-GM, GM and SynBioM bacteriophages.
76	FEFANA asbl	3.4. Information relating to the product, information relating to the production process and information relating to the product preparation process (several guidances)	? Lines 357-358: ?Full information on how the microorganism is produced must be provided (Part B, 1.2 and 3.4).? ?Full? should be replaced by ?adequate?. Requirements should be proportionate. ? Table 6, Case 5: It is unclear why for autotrophic microorganisms, updated recommendations would be required. It should be made explicit which particular aspects are not sufficiently covered by the current guidance and guidelines. ? Line 377: It is concluded that an update is needed for fermentation by auxotrophic microorganisms. We consider that the terms autotrophy and auxotrophy are interchanged here (see also our comments in the Glossary). In the case of auxotrophic microorganisms, it is noted that a number of production strains for food and feed are likely auxotrophic and such strains have been in production for a long time. If a strain	Wording “full” comes from the Directive EU 283/2013 Part B.3.4. Table 6 case 5 was adjusted as well as the conclusions. Terminology for autotrophy and auxotrophy is adjusted.

			requiring a naturally occurring compound like an amino acid, a nucleotide or a vitamin is grown in rich media, it will behave similarly to a prototroph. We would suggest that the conclusion is amended to only cover non-natural growth requirements such as non-natural amino acids or nucleotides.	
77	EuropaBio - The European Association for Bioindustries	3.4. Information relating to the product, information relating to the production process and information relating to the product preparation process (several guidances)	Lines 357-358: ?Full information on how the microorganism is produced must be provided (Part B, 1.2 and 3.4).? ?Full? should be replaced by ?adequate?. Requirements should be proportionate. Table 6, Case 5: It is unclear why for autotrophic microorganisms, updated recommendations would be required. It should be made explicit which particular aspects are not sufficiently covered by the current guidance and guidelines. Line 377: It is concluded that an update is needed for fermentation by auxotrophic microorganisms. We consider that the terms autotrophy and auxotrophy are interchanged here (see also our comments in the Glossary). In the case of auxotrophic microorganisms, it is noted that a number of production strains for food and feed are likely auxotrophic and such strains have been in production for a long time. If a strain requiring a naturally occurring compound like an amino acid, a nucleotide or a vitamin is grown in rich media, it will behave similarly to a prototroph. We would suggest that the conclusion is amended to only cover non-natural growth requirements such as non-natural amino acids or nucleotides.	idem

78	German Central Committee on Biological Safety (ZKBS)	3.4. Information relating to the product, information relating to the production process and information relating to the product preparation process (several guidances)	Some aspects of xenobionts containing XNA and/or producing xenoproteins could be addressed using the EFSA GMO guidance (2011). The information about product description, the production process as well as the product preparation process can be assessed according to the guidance. An adjustment might only be needed where information on the possible presence of recombinant DNA is collected. To include xenobionts, the term ?DNA? could be changed into ?nucleic acids?. The guidance for authorization of novel foods may also be applied.	Noted, for the context of this opinion no guidance update is envisaged by the ToR. The presence of XNA and/or xenoproteins would require the development of adapted guidance which could be based on general principles already formulated in the now existing guidances.

79	EFFCA - European Food and Feed Cultures Association	3.4. Information relating to the product, information relating to the production process and information relating to the product preparation process (several guidances)	EFFCA would like to suggest:? Lines 357-358: ?Full information on how the microorganism is produced must be provided (Part B, 1.2 ?and 3.4).? ?Full? should be replaced by ?adequate?. Requirements should be proportionate. Please note, ?that certain information like the submission of FASTA files is not a binding requirement and will be ?assessed on a case by case basis (EFSA letter, 2019, attached as reference).? In addition, EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? ? Table 6, Case 5: It is unclear why for autotrophic microorganisms, updated recommendations ?would be required. It should be made explicit which particular aspects are not sufficiently ?covered by the current guidances and guidelines.? ? Line 377: It is concluded that an update is needed for fermentation by auxotrophic ?microorganisms. We consider that the terms autotrophy and auxotrophy are interchanged here ??(see also our comments in the Glossary). In the case of auxotrophic microorganisms, it is noted ?that a number of production strains for food and feed are likely auxotrophic and such strains ?have been in production for a long time. If a strain requiring a naturally occurring compound like ?an amino acid, a nucleotide or a vitamin is grown in rich media, it will behave similarly to a ?prototroph. We would suggest that the conclusion is amended to only cover non-natural growth ?requirements such as non-natural amino acids or nucleotides.?	idem as for comment 76 (addition on FASTA files noted).

80	AMFEP	3.4. Information relating to the product, information relating to the production process and information relating to the product preparation process (several guidances)	? Lines 357-358: ?Full information on how the microorganism is produced must be provided (Part ?B, 1.2 and 3.4).? ?Full? should be replaced by ?adequate?. Requirements should be proportionate. ? ? Table 6, Case 5: It is unclear why for autotrophic microorganisms, updated recommendations ?would be required. It should be made explicit which particular aspects are not sufficiently ?covered by the current guidance and guidelines.? ? Line 377: It is concluded that an update is needed for fermentation by auxotrophic ?microorganisms. We consider that the terms autotrophy and auxotrophy are interchanged here ??(see also our comments in the Glossary). In the case of auxotrophic microorganisms, it is noted ?that a number of production strains for food and feed are likely auxotrophic and such strains ?have been in production for a long time. If a strain requiring a naturally occurring compound like ?an amino acid, a nucleotide or a vitamin is grown in rich media, it will behave similarly to a ?prototroph. We would suggest that the conclusion is amended to only cover non-natural growth ?requirements such as non-natural amino acids or nucleotides.?	idem
81	Federal Office of Consumer protection and Food Safety (BVL), National	3.5. Presence of SynBioM and SynBioM DNA or XNA in the product	Lines 392 to 396: This very valuable information together with corresponding information on available guidances /sections in other chapters should be readily summarized in a table and added to this document as an Annex Lines 398 to 400: This reference seems not to be correct. In the cited study ?Detection of antibiotic-resistant bacteria and their resistance genes from	Not followed as this is Opinion is not Guidance development and a summary of the existing guidances is out of the scope of this opinion. The reference was corrected in the reference list.

	Competent Authority		houseflies? there is no information on immunological effect of the heat-inactivated bacteria.	
82	ANSES	3.5. Presence of SynBioM and SynBioM DNA or XNA in the product	No comment	na
83	Evonik Operations GmbH	3.5. Presence of SynBioM and SynBioM DNA or XNA in the product	Lines 398-400: Please check the reference Akter et al. (2020) in regard to your statement ?for heat-inactivated bacteria have been demonstrated to have effects on the immunological functions of the exposed humans and animals? and please cite suitable literature. This article is about ?Detection of antibiotic-resistant bacteria and their resistance genes from houseflies?. Lines 408-409: The presence of DNA is not a hazard according to the definition provided in lines 1537-1540 ?hazard is the potential of an organism?. DNA is not an organism and not a biological entity that is capable of replicating outside of the organism. Lines 409-410: Delete the sentence ?Moreover, the presence of DNA from the GMM is the determining criterion whether the product falls or not under the EU GMO regulation.? See also comments under Section 2.4 above.	The reference was corrected in the reference list. The hazard related to the presence of DNA is explained in the light of horizontal gene transfer of genes of concern. The sentence referring to the legislation has been deleted.

84	FEFANA asbl	3.5. Presence of SynBioM and SynBioM DNA or XNA in the product	? Line 389: Absence of DNA is not a regulatory requirement for fermentation products, as determined in a recent legal opinion (Dederer, 2021; https://stoffr.lexxion.eu/article/STOFFR/2021/3/6 ; see also section 2.4). Therefore, the text should be amended as follows (see section 5.4): ?It also may not contain DNA with sequences of concern or XNA. Category 3 products may contain DNA with sequences of concern or XNA but no [?]? ? Line 391: It should be clarified that the mere presence of DNA from the production strains is not a safety risk. It is the potential presence of genes of concern (such as AMR genes, pathogenicity factors, toxins). A more differentiated approach to the requirements for the absence of DNA from the production strains, depending on the presence/absence of genes of concern could be suggested in future guidance. ? Lines 398-400: Please check the reference Akter et al. (2020) and please cite suitable literature. This article is about ?Detection of antibiotic-resistant bacteria and their resistance genes from houseflies?. ? Lines 408-409: The presence of DNA is not a hazard according to the definition in lines 1537-1540. DNA is not an organism nor a biological entity capable of replicating outside of the organism. ? Lines 409-410: Delete the sentence ?Moreover, the presence of DNA from the GMM is the determining criterion whether the product falls or not under the EU GMO regulation.? See also comments under Section 2.4. ? Lines 411-415: Regarding the description of the	Idem as response to comment 83. Details on methods for demonstrating the presence of DNA are reported in the specific guidance.

			detection, the current guidance and current LODs are adequate for the requirements on absence of DNA of the production strain. Since the method (PCR), choice of gene length and number of genes to detect is mentioned, the current LOD should be stated as well. A sentence could be added in line 415: ?The currently stated LOD of 10 ng/g or 10 ng/ml is sufficient for the safety evaluation of production strains containing sequences of concern that are used for products of categories 1 and 2.?	
85	EuropaBio - The European Association for Bioindustries	3.5. Presence of SynBioM and SynBioM DNA or XNA in the product	Line 389: Absence of DNA is not a regulatory requirement for fermentation products, as determined in a recent legal opinion (Dederer, 2021; https://stoffr.lexxion.eu/article/STOFFR/2021/3/6 ; see also section 2.4). Therefore, the text should be amended as follows (see also section 5.4): ?It also may not contain DNA with sequences of concern or XNA. Category 3 products may contain DNA with sequences of concern or XNA but no [?]? Line 391: In addition, it should be clarified that the mere presence of DNA from the production strains is not a safety risk. It is the potential presence of genes of concern (such as AMR genes, pathogenicity factors, toxins). In this regard, a more differentiated approach to the requirements for the absence of DNA from the production strains, depending on the presence/absence of genes of concern could be suggested in future guidance. Lines 398-400: Please check the reference Akter et al. (2020) and cite suitable literature. This article is about	idem

			?Detection of antibiotic-resistant bacteria and their resistance genes from houseflies? Lines 408-409: The presence of DNA is not a hazard according to the definition in lines 1537-1540. DNA is not an organism nor a biological entity capable of replicating outside of the organism. Lines 409-410: Delete ?Moreover, the presence of DNA from the GMM is the determining criterion whether the product falls or not under the EU GMO regulation.? See also comments under Section 2.4. Lines 411-415: Regarding the description of the detection, the current guidance and current LODs are adequate for the requirements on absence of DNA of the production strain. Since the method (PCR), choice of gene length and number of genes to detect is mentioned, the current LOD should be stated as well. A sentence could be added in line 415: ?The currently stated LOD of 10 ng/g or 10 ng/ml is sufficient for the safety evaluation of production strains containing sequences of concern that are used for products of categories 1 and 2."	
86	AMFEP	3.5. Presence of SynBioM and SynBioM DNA or XNA in the product	? Line 389: Absence of DNA is not a regulatory requirement for fermentation products, as ?determined in a recent legal opinion (Dederer, 2021; ?https://stoffr.lexxion.eu/article/STOFFR/2021/3/6; see also section 2.4). The text should be ?amended as follows (see also section 5.4): ?It also may not contain DNA with sequences of ?concern or XNA. Category 3 products may contain DNA with sequences of concern or XNA but no ??[?]?? ? Line 391: it should be clarified	idem

			that the mere presence of DNA from the production strains is not ?a safety risk. It is the potential presence of genes of concern (such as AMR genes, pathogenicity ?factors, toxins). In this regard, a more differentiated approach to the requirements for the ?absence of DNA from the production strains, depending on the presence/absence of genes of ?concern could be suggested in future guidance.? ? Lines 398-400: Please check the reference Akter et al. (2020) and cite suitable literature. This ?article is about ?Detection of antibiotic-resistant bacteria and their resistance genes from ?houseflies?? ? Lines 408-409: The presence of DNA is not a hazard according to the definition in lines 1537-??1540. DNA is not an organism nor a biological entity capable of replicating outside of the ?organism.? ? Lines 409-410: Delete ?Moreover, the presence of DNA from the GMM is the determining ?criterion whether the product falls or not under the EU GMO regulation.? See also comments ?under Section 2.4.? ? Lines 411-415: on the description of the detection, the current guidance and LODs are adequate ?for the requirements on absence of DNA of production strain. Since the method (PCR), choice of ?gene length and number of genes to detect is mentioned, the current LOD should be also stated. ?A sentence could be added in line 415: ?The currently stated LOD of 10 ng/g or 10 ng/ml is ?sufficient for the safety evaluation of production strains containing sequences of concern that are ?used for products of categories 1 and 2."?	

87	Testbiotech	3.5. Presence of SynBioM and SynBioM DNA or XNA in the product	Already there is an increasing number of alerts regarding the unintended presence of DNA or viable genetically engineered organisms in food and feed products registered in the RASFF. Therefore, guidance for detection and identification of the unintended presence of SynBioM and DNA or XNA should be developed urgently (including the proposed categories 1 and 2).	Noted, this comment is out of scope and the ToR for this opinion, see comment 47 for detection.
88	ANSES	3.6. Comparative approach of the EFSA GMO Panel GMM guidance 2011	No comment	na
89	ANSES	3.7. Toxicology	On which basis are currently assessed non GM-bacteriophages, if no specific guidance exist ? Can a pathogenic strain be used to propagate the phage? In human phage therapy perhaps ?	Noted, this comment is out of scope and the ToR for this opinion.
90	FEFANA asbl	3.7. Toxicology	? With regard to the statement made in lines 508-511 we suggest clarifying that only acquired AMR genes (or antibiotic resistance marker genes) are of importance, while intrinsic AMR genes are not relevant in this regard. This will ensure alignment with current EFSA Guidance as well as a risk driven evaluation of organisms. ? Lines 506-508: The lowest taxonomic unit to which QPS status is assigned currently is the species. We recommend expanding the potential for exemptions also to the sub-species level (e.g. E. coli K12) and to lineages of strains with scientifically demonstrated ?intrinsic? safety (see also section 3.3. above).	EFSA considers the sentence " but harbours acquired AMR genes" is sufficiently clear and reflects what is reported in the most recent guidance. Idem as comment 69: EFSA notes the suggestion made for extending the QPS approach to certain lineages (which is outside the scope of this opinion).

91	EuropaBio - The European Association for Bioindustries	3.7. Toxicology	With regard to the statement made in lines 508-511 we suggest clarifying that only acquired AMR genes (or antibiotic resistance marker genes) are of importance, while intrinsic AMR genes are not relevant in this regard. This will ensure alignment with current EFSA Guidance as well as a risk driven evaluation of organisms. Lines 506-508: The lowest taxonomic unit to which QPS status is assigned currently is the species. We recommend expanding the potential for exemptions also to the sub-species level (e.g. E. coli K12) and to lineages of strains with scientifically demonstrated ?intrinsic? safety (see also section 3.3. above).	idem
92	EFFCA - European Food and Feed Cultures Association	3.7. Toxicology	EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? ? With regard to the statement made in lines 508-511 we suggest to clarify, that only acquired ?AMR genes (or antibiotic resistance marker genes) are of importance, while intrinsic AMR genes ?are not relevant in this regard. This will ensure alignment with current EFSA Guidance as well as a ?risk driven evaluation of organisms.? ? Lines 506-508: The lowest taxonomic unit to which QPS status is assigned currently is the ?species. We recommend expanding the potential for exemptions also to the sub-species level ??(e.g. E. coli K12) and to lineages of strains with scientifically demonstrated ?intrinsic? safety (see ?also section 3.3. above).?	idem

93	AMFEP	3.7. Toxicology	? With regard to the statement made in lines 508-511 we suggest clarifying that only acquired ?AMR genes (or antibiotic resistance marker genes) are of importance, while intrinsic AMR genes ?are not relevant in this regard. This will ensure alignment with current EFSA Guidance as well as a ?risk driven evaluation of organisms.? ? Lines 506-508: The lowest taxonomic unit to which QPS status is assigned currently is the ?species. We recommend expanding the potential for exemptions also to the sub-species level ??(e.g. E. coli K12) and to lineages of strains with scientifically demonstrated ?intrinsic? safety (see ?also section 3.3. above).?	idem
94	Testbiotech	3.7. Toxicology	Microorganisms which are supposed to produce biologically active molecules, such as non-coding RNAs (ncRNAs), to influence the characteristics of food-producing animals (such as bees or livestock) or plants (for example via micorrhiza or endobionts), which may thereby enter the food production chain directly or indirectly will need specific attention. These issues go beyond what is indicated for plant protection products, and the need for updated guidelines does not only concern exposure. The QPS also needs to be assessed in regard to an evaluation of the QPS concept for (i) unintended interactions within the cells and (ii) potential interactions, accumulated and combinatorial effects between organisms caused by the (intended or unintended) presence of more than one SynBioM in a specific environment. Furthermore, new guidelines are needed on how to generally include the potential impacts and interactions of accumulated and	Non-coding RNA cases: idem as above comment 40. QPS and interactions: idem as above comment 73 Combinatorial effects: idem as above comment 47

			combinatorial effects caused by the presence of more than one SynBioM in a joint environment, by taking into account specific scenarios.	
95	Federal Office of Consumer protection and Food Safety (BVL), National Competent Authority	3.8. Gut microbiome and horizontal gene transfer	see uploaded file	Comments are addressed in Appendix B.
96	ANSES	3.8. Gut microbiome and horizontal gene transfer	In general, knowledge of complex microbial ecosystems is only at its beginning. We know how to identify the dominant flora and the identification of these flora at the species level is often not yet precise. As regards the sub-dominant flora, knowledge is even more rudimentary. Moreover, the interactions between the microorganisms constituting these flora are far from being known, as well as their impact on the host. It seems difficult in the current state of these scientific deficiencies to predict the future and the impact of GMMs and more specifically of SymBioMs in the ecosystems in which they will evolve. L539 : Acronym explained before (p11) in a table. Perhaps recall its meaning here ? L621 : As long as knowledge of the intestinal microbiome is fragmentary (identification of all species and not of families and interactions between these species and interactions between these species and the human or animal organism), the use of such	Noted. Risk Assessment for a given product is to be delivered on the basis of the knowledge that is currently available and includes possible interactions with the environment (see Opinion 1 https://www.efsa.europa.eu/en/efsajournal/pub/6263). Comment on acronym is unclear. The suggestion for prohibition is outside the RA remit. State of the art and potential needs identified, are included in the text of this opinion. Line 662 - Text was adjusted.

			GMMs should be prohibited. L662 : How can these categories have an effect on the microbiota ?	
97	Evonik Operations GmbH	3.8. Gut microbiome and horizontal gene transfer	Table 10, Cases 4, 5 and 6: the recommendation that ?updates [are] needed on methodology to assess on impact on the microbiome structure and functionality?. The first step should be to substantiate the perceived risks here. Only if this truly materializes as a risk will additionally risk assessment measures be required. In the text (lines 670-671) this is confirmed: ?No consensus exists as to what is a healthy baseline in the analysis of gut microbiota.? No reference material of the original status exists so far, and the question is unanswered how far away from the original state of gut microbiome is an unwanted change or a natural fluctuation, both in structure and functionality. Lines 646-647: In general, it could be that the use of bioinformatics analysis for measuring HGT potential is equally applicable to GMMs and SynBioMs, but the evaluation of the extent of identity between GM event searches (in most cases microbial sequences) and the DNA present in microbial genomes is more complex and far less clear.	Noted. State of the art and potential needs identified, are included in the text of this opinion.

98	FEFANA asbl	3.8. Gut microbiome and horizontal gene transfer	? Table 10, Cases 4, 5 and 6: the recommendation that ?updates [are] needed on methodology to assess on impact on the microbiome structure and functionality?. The first step needs to be to substantiate the perceived risks here. Only if this truly materializes as a risk would additional risk assessment measures be required. This view is also supported by lines 670-671. In addition, no reference material of the original status exists, and the question is unanswered how far away from the original state of gut microbiome is an unwanted change or a natural fluctuation, both in structure and functionality. The topic of gut microbiome is in the bird watching state, no methods of analysis and evaluation exist and should not be considered for risk assessment purposes. ? Lines 646-647: In general, it could be that the use of bioinformatics analysis for measuring HGT potential is equally applicable to GMMs and SynBioMs, but the evaluation of the extent of identity between GM event searches (in most cases microbial sequences) and the DNA present in microbial genomes is more complex and far less clear. ? Lines 672-686: Before any efficiently designed and evaluated methodologies to assess the impact of any products on the gut microbiome can be developed, it must first be clearly defined what a healthy gut microbiome is. There is currently no accepted definition. Moreover, we want to point out that non-GM organisms or new-to-nature compounds may not be in the scope of this evaluation and should be excluded. Further, establishment of such	Idem as comment 96: Noted. State of the art and potential needs identified, are included in the text of this opinion. HGT: text slightly adjusted. The QPS opinion explains the approach to safety assessment, which is applicable to GMM and SynBioM when the species is included I the QPS list and when the genetic modification would not cause a concern. This concept is also applicable to effects on gut microbiome. Text adjusted and replaced by explored.

			methodologies might lead to increased requirements for products which can sufficiently be assessed following the current guidance. With regard to QPS strains, it should be clearly stated that such strains would not require additional studies on the gut microbiome since they are already considered safe. ? Line 677: please replace ?designed? by ?explored?, as the science does not seem to be established sufficiently to allow ?design?.	
99	EuropaBio - The European Association for Bioindustries	3.8. Gut microbiome and horizontal gene transfer	Table 10, Cases 4, 5 and 6: the recommendation that ?updates [are] needed on methodology to assess on impact on the microbiome structure and functionality?. The first step needs to be to substantiate the perceived risks here. Only if this truly materializes as a risk would additional risk assessment measures be required. This view is also supported by lines 670-671. In addition, no reference material of the original status exists, and the question is unanswered how far away from the original state of gut microbiome is an unwanted change or a natural fluctuation, both in structure and functionality. The topic of gut microbiome is in the bird watching state, no methods of analysis and evaluation exist and should not be considered for risk assessment purposes. Lines 646-647: In general, it could be that the use of bioinformatics analysis for measuring HGT potential is equally applicable to GMMs and SynBioMs, but the evaluation of the extent of identity between GM event searches (in most cases microbial sequences) and the DNA present in microbial genomes is more complex	idem

			and far less clear. Lines 672-686: Before any efficiently designed and evaluated methodologies to assess the impact of any products on the gut microbiome can be developed, it must first be clearly defined what a healthy gut microbiome is. There is currently no accepted definition. Moreover, we want to point out that non-GM organisms or new-to-nature compounds may not be in the scope of this evaluation and should be excluded. Further, establishment of such methodologies might lead to increased requirements for products which can sufficiently be assessed following the current guidance. With regard to QPS strains, it should be clearly stated that such strains would not require additional studies on the gut microbiome since they are already considered safe. Line 677: please replace ?designed? by ?explored?, as the science does not seem to be established sufficiently to allow ?design?.	
100	German Central Committee on Biological Safety (ZKBS)	3.8. Gut microbiome and horizontal gene transfer	The box framing lines 592-620 is not referring to the case studies identified. Instead it highlights microorganisms that could gain their specific functions through a classical genetic engineering approach as well. This applies to all examples stated in the box: microorganisms with increased adhesion abilities, microorganisms producing specific metabolites, phages designed to inhibit enteropathogens or strains engineered to boost the immune system. Furthermore, the latter two examples are medical and not food and feed applications. The section should be adapted to the other sections of the document and concentrate	Noted. The text of the box was rearranged. Terminology exchange: see comment 46.

			on the case studies, or even better, suitable case studies should be selected. As the section highlights correctly, effects on the microbiome have to be studied for all kinds of products, independent from their non-GM, GMM or SynBioM status. A differentiation between these product categories is not necessary. The EFSA GMO guidance (2011) may be applicable to xenobionts containing XNA and/or producing xenoproteins, if the term ?DNA? is exchanged with ?nucleic acids?.	
101	AMFEP	3.8. Gut microbiome and horizontal gene transfer	? Table 10, Cases 4, 5 and 6: the recommendation that ?updates [are] needed on methodology to ?assess on impact on the microbiome structure and functionality?. The first step needs to be to ?substantiate the perceived risks here. Only if this truly materializes as a risk would additional risk ?assessment measures be required. This view is also supported by lines 670-671. In addition, no ?reference material of the original status exists, and the question is unanswered how far away ?from the original state of gut microbiome is an unwanted change or a natural fluctuation, both in ?structure and functionality. The topic of gut microbiome is in the bird watching state, no ?methods of analysis and evaluation exist and should not be considered for risk assessment ?purposes.? ? Lines 646-647: In general, it could be that the use of bioinformatics analysis for measuring HGT ?potential is equally applicable to GMMs and SynBioMs, but the evaluation of the extent of ?identity between GM event searches (in most cases microbial sequences) and the DNA	idem to 98

			present in ?microbial genomes is more complex and far less clear.? ? Lines 672-686: Before any efficiently designed and evaluated methodologies to assess the impact ?of any products on the gut microbiome can be developed, it must first be clearly defined what a ?healthy gut microbiome is. There is currently no accepted definition. Moreover, we want to point ?out that non-GM organisms or new-to-nature compounds may not be in the scope of this ?evaluation and should be excluded. Further, establishment of such methodologies might lead to ?increased requirements for products which can sufficiently be assessed following the current ?guidance. With regard to QPS strains, it should be clearly stated that such strains would not ?require additional studies on the gut microbiome since they are already considered safe.? ? Line 677: replace ?designed? by ?explored?, as the science does not seem to be established ?sufficiently to allow ?design?.?	
102	Testbiotech	3.8. Gut microbiome and horizontal gene transfer	The discussion of effects on and via the microbiome should be widened to include the more general concept of the holobiont. Without such a concept, anyrisk assessment guidance will remain fragmentary. It should not only include the microbial communities in the gut, but also other interactive networks, such as including the skin, the respiratory system and the rhizome, which may all be impacted by the intended or unintended presence of SynBioM. The selection of cases should also integrate SynBioM which produce biologically active substances, such	The holobiont concept is currently far from practical implementation. However, it is implicitly addressed in the opinion where interactions between microorganisms and the host are dealt with. Non coding RNA cases: Idem to comment 40 and 41. Combinatorial effects: idem as comments 47. Noted. This is not specifically related to Synthetic Biology but to all risk assessments. Case-by-case assessment per product is currently foreseen in

			ncRNAs, to alter the composition of the microbiome intentionally, or to change the biological characteristics of the host. The discussion of effects on and via the microbiome should also include the unintended presence of SynBioM which may interact with microbial communities. Furthermore, new guidelines are needed on how to generally include the potential impacts and interactions of accumulated and combinatorial effects caused by the presence of more than one SynBioM in a joint environment, by taking into account specific scenarios.	legal frameworks, which are implemented by EFSA RAs.
103	POLLINIS	3.8. Gut microbiome and horizontal gene transfer	Current work on the microbiota and in the honey bee gut is advancing quickly [5-10]. Borum (2021) states: ?the microbiota has important functions in metabolism, immune system, growth and development? Microbiata species can alter both the volatile profiles and olfactory behaviours of the host?. Thus, the microbiota affects the honey bee?s memory and learning capacity ? and therefore its neurophysiological development via foraging, mating and chemical communication. It is important for EFSA to consider the microbiota in a more broader way to include not only the microbiota itself but the organism as a whole and the context of the organism in which it is surrounded. This idea is emphasised in notion of holobiont. As Rosenberg & Zilber-Rosenberg states: ?Microbiotas and their hosts interact in a manner that affects the fitness of the holobiont in many ways, including its morphology, development, behavior, physiology, and resistance to disease. Taken together, these	Honey Bee: see response to comment 40 and 41 with the introduction of nex case studies in the text. Holobiont: see above response for comment 102. Horizontal gene transfer in the environment is out of the scope of this opinion and is (for SynBioM) already addressed in Opinion 1 (https://www.efsa.europa.eu/en/efsajournal/pub/6263).

			interactions characterize the holobiont as a single and unique biological entity? (pg. 1) [11]. The effects of microorganism to the rest of the organism in which it is inserted, or its surrounding environment must be considered in any risk assessment. Without such consideration might weaken the risk assessment. Moreover, we urge EFSA to include in its guidance a recent article by Xia et al. (2021) who demonstrated the first example of a natural gene transfer from a plant to an insect [12]. Considering the importance of current and potential work of the microbiota and the honey bee, and a real possibility of horizontal gene transfer between plant and insect push forward the relevance for need to broaden this risk assessment.	
104	ANSES	3.9. Allergenicity	Replace ?degree of sequence homology? by ?degree of sequence identity? because sequence homology has a similar sense as sequence similarity, that is a distinct sense from sequence identity. Replace by ?using a sliding window of 80 amino acids with a cut off > 35% identity and a sliding window of 8 amino acids with a cut off of 100% identity, analysis?.? - Does the reference to clinical tests, as it is introduced, refer to an experimental approach which has to be added to the weight of evidence approach for evaluating the allergenicity? or it simply means that clinical tests should be performed if data resulting from the weight of evidence approach suggest a potential allergic risk? This sentence is confusing and should be rewritten to clarify the position of EFSA on this point. An additional point concerns	Text has been changed on these various points.

			the lack of standardized IgE to evaluate the IgE-binding capacity of potential allergenic proteins, e.g. in ELISA tests, because the affinity, specificity and polyclonal character, play a key role and can largely modify the ELISA test results. ELISA instead of ?elisa?? This is an abbreviation, not a birthname. The in vitro SGF and SIF are performed to get some insight into the resistance of proteins to digestive proteases, which is indirectly related to their digestibility (ability to be digested in physiological conditions). The question of the route of sensitization for potentially allergenic proteins is of paramount importance and should be evaluated on a case-by-case basis, depending on the nature (enzymes or not), physico-chemical properties (resistance to heat denaturation and digestive proteases). In this respect, the size of peptides resulting from the action of digestive proteases on the proteins, is of paramount importance because allergenic potentialities are attributed to peptides exhibiting a sufficient length, usually > 10 amino acids. The position of EFSA on the evaluation of new proteins has to be toned down because most of the widely distribut	
105	FEFANA asbl	3.9. Allergenicity	? Table 11, Case 12: ?Lactococcus chassis? should be replaced by ?Lactococcus lactis? ? Line 739: ?developed? should be replaced by ?explored?, reflecting the current status of science on this topic. ? Lines 739-740: What also should be explored are in vitro methods for skin sensitisation, skin irritation and eye irritation that work with microorganisms and other proteinaceous substances.	Text adjusted where considered correct and when in scope of this opinion.

106	EuropaBio - The European Association for Bioindustries	3.9. Allergenicity	Table 11, Case 12: ?Lactococcus chassis? should be replaced by ?Lactococcus lactis? Line 739: ?developed? should be replaced by ?explored?, reflecting the current status of science on this topic. Lines 739-740: What also should be explored are in vitro methods for skin sensitisation, skin irritation and eye irritation that work with microorganisms and other proteinaceous substances.	idem
107	EFFCA - European Food and Feed Cultures Association	3.9. Allergenicity	EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? ? Table 11, Case 12: ?Lactococcus chassis? should be replaced by ?Lactococcus lactis?? ? Line 739: ?developed? should be replaced by ?explored?, reflecting the current status of science ?on this topic.? ? Lines 739-740: What also should be explored are in vitro methods for skin sensitisation, skin ?irritation and eye irritation that actually work with microorganisms and other proteinaceous ?substances.?	idem
108	AMFEP	3.9. Allergenicity	? Table 11, Case 12: ?Lactococcus chassis? should be replaced by ?Lactococcus lactis?? ? Line 739: ?developed? should be replaced by ?explored?, reflecting the current status of science ?on this topic.? ? Lines 739-740: What also should be explored are in vitro methods for skin sensitisation, skin ?irritation and eye irritation that work with microorganisms and other proteinaceous substances.?	idem
109	Testbiotech	3.9. Allergenicity	New guidelines are needed on how to generally include the potential impacts and interactions of accumulated and combinatorial effects caused by the presence of more than one SynBioM in a joint	Idem as response to comment 47. In addition, allergenicity is highly allergen specific. Apart from cross reactivity, combinatorial effects of allergens as such is not expected. Perhaps what is

			environment, by taking into account specific scenarios. New guidance is needed on how to detect, identify, monitor and control the unintended presence of SynBioM and their DNA or XNA.	meant here is potential adjuvant effects. This is already covered in the text.
110	Testbiotech	4. Outlook ? Phase 3 evaluation	In view of plans to introduce, without any precedence, a high number of SynBioM with different traits and from different species into the environment (and into the food and feed chain), inheriting biological characteristics that go far beyond what was achieved by previous methods, a concerted effort is needed to develop internationally agreed guidance and harmonised frameworks to strengthen the precautionary principle. These efforts should take into account: ? Prospective technology assessment and horizon screening; ? Guidance on how to integrate scenarios that include potential impacts and interactions of accumulated and combinatorial effects caused by the presence of more than one SynBioM in a joint environment; ? The biological concept of the holobiont - taking into account that the risks of SynBioM cannot be assessed simply by looking at single cells in isolation; ? Guidance on how to effectively limit and control the overall the release of SynBioM into the environment in regard to numbers, different traits and species; ? Guidance on how to apply effective cut-off criteria in the face of non-knowledge, a high degree of uncertainties and non-conclusive risk assessment; ? Guidance which includes detection and methods to monitor the unintended presence of SynBioM and their DNA or XNA in the food chain and the	Noted. This comment on the precautionary principle is outside of the scope of the ToR of this opinion. See various responses above: Combinatorial effects and detection: idem as for comment 47. Holobiont: idem as for comment 102. QPS: idem as for comment 73

			environment; ? An evaluation of the QPS concept in regard to (i) unintended interactions within the cells as well as (ii) new phenotypical characteristics of the cell populations within one species (such as growth of bacteria) and (iii) potential interactions, accumulated and combinatorial effects between organisms caused by the (intended or unintended) presence of more than one SynBioM in a specific environment.	
111	POLLINIS	4. Outlook ? Phase 3 evaluation	Based on the current guidelines, it seems that EFSA is planning to introduce microorganisms obtained through synthetic biology, especially through food and feed, food for humans (e.g. additives, decontaminants) going outside previous methods. Not only do some of these introductions have a history of safe use, many of the considerations of unintended or intended effects have not been adequately considered. We think it is a good idea that EFSA prioritise the precautionary principle developing international guidelines that take into account: - To better understand the unintended and intended effects of applications of synthetic biology - Investigate how to develop a technological assessment and horizon screening - Incorporate a better and more holistic understanding of microorganism, its host and its environment	Noted. This comment is outside of the scope of the ToR of this opinion. Risk management issues are outside of the remit of EFSA.

112	Evonik Operations GmbH	4.1. Tiered approach for risk assessment of living cells ingested by humans/animals	Lines 892-894: We fully support that global harmonization is key. In addition, future guidance should be based on solid, validated scientific evidence. We would very much appreciate if EFSA could indicate in this section how such global harmonization will be pursued, and how it will be avoided that EFSA will unilaterally introduce new requirements ahead of an alignment with other regions.	Noted. This comment is outside of the scope of the ToR of this opinion.
113	FEFANA asbl	4.1. Tiered approach for risk assessment of living cells ingested by humans/animals	? Lines 892-894: We fully support that global harmonization is key. In addition, future guidance should be based on solid, validated scientific evidence. We would very much appreciate if EFSA could indicate in this section how such global harmonization will be pursued, and how it will be avoided that EFSA will unilaterally introduce new requirements ahead of an alignment with other regions. ? We appreciate the concerns with regard to the potential impact of living cell intake. This should be closely related to the risk associated with the respective microorganism. Further, QPS strains should not be investigated in this way, as they are considered safe already. This should be clearly stated in this context.	Idem as for comment 112. Noted. QPS is an EFSA/EU instrument to be applied with qualifications on a case by case basis.

114	EuropaBio - The European Association for Bioindustries	4.1. Tiered approach for risk assessment of living cells ingested by humans/animals	Lines 892-894: We fully support that global harmonization is key. In addition, future guidance should be based on solid, validated scientific evidence. We would very much appreciate if EFSA could indicate in this section how such global harmonization will be pursued, and how it will be avoided that EFSA will unilaterally introduce new requirements ahead of an alignment with other regions. We appreciate the concerns with regard to the potential impact of living cell intake. This should be closely related to the risk associated with the respective microorganism. Further, QPS strains should not be investigated in this way, as they are considered safe already. This should be clearly stated in this context.	idem
115	EFFCA - European Food and Feed Cultures Association	4.1. Tiered approach for risk assessment of living cells ingested by humans/animals	EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? ? Lines 892-894: We fully support that global harmonization is key. In addition, future guidance ?should be based on solid, validated scientific evidence. We would very much appreciate if EFSA ?could indicate in this section how such global harmonization will be pursued, and how it will be ?avoided that EFSA will unilaterally introduce new requirements ahead of an alignment with other ?regions.? ? We appreciate the concerns with regard to the potential impact of living cell intake. This should ?be closely related to the risk associated with the respective microorganism. Further, QPS strains ?should not be investigated in this way, as they are considered safe already. This should be clearly ?stated in this context.?	idem

116	AMFEP	4.1. Tiered approach for risk assessment of living cells ingested by humans/animals	? Lines 892-894: We fully support that global harmonization is key. In addition, future guidance ?should be based on solid, validated scientific evidence. We would very much appreciate if EFSA ?could indicate in this section how such global harmonization will be pursued, and how it will be ?avoided that EFSA will unilaterally introduce new requirements ahead of an alignment with other ?regions.? ? We appreciate the concerns with regard to the potential impact of living cell intake. This should ?be closely related to the risk associated with the respective microorganism. Further, QPS strains ?should not be investigated in this way, as they are considered safe already. This should be clearly ?stated in this context.?	idem
117	Evonik Operations GmbH	4.2. Evolving from a technique-driven risk assessment approach towards a strain-driven approach	It is acknowledged in this section that a process-based risk assessment is more and more challenging and a product-based risk assessment on the WGS would be more appropriate. The long-term perspective for innovative biotechnology in Europe should also consider a significant modernization of the EU?s GMO legislation more broadly. This legislation should be based on the characteristics of the organisms rather than on the technologies used to develop them.	Noted. This comment is outside of the scope of the ToR of this opinion.

118	FEFANA asbl	4.2. Evolving from a technique-driven risk assessment approach towards a strain-driven approach	? Lines 903-906: This sentence is a bit difficult to understand. Please confirm and clarify further that for the future safety assessment for food and feed use, a comparison with the non-genetically modified counterpart will not be necessary anymore. The future risk assessment will be based solely on the organism in question. An example for the amended version could be: ?Therefore, the RA of GMMs is already now developing towards an approach based on the assessment of the genetic composition of the GMM, independently of the genetic modification techniques used. Likewise, for the assessment of the safety for food and feed use, RA is now developing towards an approach independent of the non-genetically modified counterpart.? ? It is acknowledged in this section that a process-based risk assessment is more and more challenging and a product-based risk assessment on the WGS would be more appropriate. The long-term perspective for innovative biotechnology in Europe should also consider a significant modernization of the EU?s GMO legislation more broadly. This legislation should be based on the characteristics of the organisms rather than on the technologies used to develop them. ? Moreover, we suggest elaborating more on the consequences for products in categories 1-2 (or the future merged category) and clearly state again that for these cases the risk assessment should be performed following the specific regulations as stated in Figure 1, which are still adequate for the present and the future.	The section of the opinion has been clarified. Legislation: This comment is outside of the scope of the ToR of this opinion. Figure 1 is deleted.

119	EuropaBio - The European Association for Bioindustries	4.2. Evolving from a technique-driven risk assessment approach towards a strain-driven approach	Lines 903-906: This sentence is a bit difficult to understand. Please confirm and clarify further that for the future safety assessment for food and feed use, a comparison with the non-genetically modified counterpart will not be necessary anymore. The future risk assessment will be based solely on the organism in question. An example for the amended version could be: ?Therefore, the RA of GMMs is already now developing towards an approach based on the assessment of the genetic composition of the GMM, independently of the genetic modification techniques used. Likewise, for the assessment of the safety for food and feed use, RA is now developing towards an approach independent of the non-genetically modified counterpart.? It is acknowledged in this section that a process-based risk assessment is more and more challenging and a product-based risk assessment on the WGS would be more appropriate. The long-term perspective for innovative biotechnology in Europe should also consider a significant modernization of the EU?s GMO legislation more broadly. This legislation should be based on the characteristics of the organisms rather than on the technologies used to develop them. Moreover, we suggest elaborating more on the consequences for products in categories 1-2 (or the future merged category) and clearly state again that for these cases the risk assessment should be performed following the specific regulations as stated in Figure 1, which are still adequate for the present and the future.	idem

120	German Central Committee on Biological Safety (ZKBS)	4.2. Evolving from a technique-driven risk assessment approach towards a strain-driven approach	The ZKBS welcomes the recommendation to envisage a strain-driven risk assessment approach instead of a technique-driven approach. Furthermore, the ZKBS considers it necessary to follow a case-by-case risk assessment as it is already provided in ?EFSA GMO Panel, 2011 ? Guidance on the risk assessment of GMMs and their products intended for food and feed use?.	Noted.
121	EFFCA - European Food and Feed Cultures Association	4.2. Evolving from a technique-driven risk assessment approach towards a strain-driven approach	EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? ? Lines 903-906: This sentence is a bit difficult to understand. Please confirm and clarify further ?that for the future safety assessment for food and feed use, a comparison with the non-?genetically modified counterpart will not be necessary anymore. The future risk assessment will ?be based solely on the organism in question. An example for the amended version could be: ???Therefore, the RA of GMMs is already now developing towards an approach based on the ?assessment of the genetic composition of the GMM, independently of the genetic modification ?techniques used. Likewise, for the assessment of the safety for food and feed use, RA is now ?developing towards an approach independent of the non-genetically modified counterpart.?? ? It is acknowledged in this section that a process-based risk assessment is more and more ?challenging and a product-based risk assessment on the WGS would be more appropriate. The ?long-term perspective for innovative biotechnology in Europe should also consider a significant ?modernization of the EU?s GMO legislation more	Idem as response to comment 118

			broadly. This legislation should be based on the ?characteristics of the organisms rather than on the technologies used to develop them.? ? Moreover, we suggest elaborating more on the consequences for products in categories 1-2 (or ?the future merged category) and clearly state again that for these cases the risk assessment ?should be performed following the specific regulations as stated in Figure 1, which are still ?adequate for the present and the future.?	
122	AMFEP	4.2. Evolving from a technique-driven risk assessment approach towards a strain-driven approach	? Lines 903-906: This sentence is a bit difficult to understand. Please confirm and clarify further ?that for the future safety assessment for food and feed use, a comparison with the non-?genetically modified counterpart will not be necessary anymore. The future risk assessment will ?be based solely on the organism in question. An example for the amended version could be: ???Therefore, the RA of GMMs is already now developing towards an approach based on the ?assessment of the genetic composition of the GMM, independently of the genetic modification ?techniques used. Likewise, for the assessment of the safety for food and feed use, RA is now ?developing towards an approach independent of the non-genetically modified counterpart.?? ? It is acknowledged in this section that a process-based risk assessment is more and more ?challenging and a product-based risk assessment on the WGS would be more appropriate. The ?long-term perspective for innovative biotechnology in Europe should also consider a significant ?modernization of the EU?s GMO legislation more	idem

			broadly. This legislation should be based on the ?characteristics of the organisms rather than on the technologies used to develop them.? ? Moreover, we suggest elaborating more on the consequences for products in categories 1-2 (or ?the future merged category) and clearly state again that for these cases the risk assessment ?should be performed following the specific regulations as stated in Figure 1, which are still ?adequate for the present and the future?	
123	FEFANA asbl	5.1. Identification of newer sectors/advances	? Lines 932-933: It is important to clarify those regulations and requirements focusing on SynBioMs will likely also affect GMM which were not intended to be in the scope. This could be further clarified in the section. ? Line 935: Please see our comments on ?extensively engineered? under the Glossary section below.	In the conclusions of the Opinion a differentiation is made when the conclusion and the need for updates is applying to SynBioM or when applying to broader microorganism types (non-GM, GMM or SynBioM). The term “extensively engineered” is deleted.
124	EuropaBio - The European Association for Bioindustries	5.1. Identification of newer sectors/advances	Lines 932-933: It is important to clarify those regulations and requirements focusing on SynBioMs will likely also affect GMM which were not intended to be in the scope. This could be further clarified in the section. Line 935: Please see our comments on ?extensively engineered? under the Glossary section below.	idem
125	EFFCA - European Food and Feed Cultures Association	5.1. Identification of newer sectors/advances	EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? ? Especially the lines 932-933 are important to make clear that regulations and requirements ?which might be created focusing SynBioMs will likely also affect GMM which were not intended	idem

			?to be in the scope. This could be further clarified in the section.?	
126	AMFEP	5.1. Identification of newer sectors/advances	? Lines 932-933: It is important to clarify those regulations and requirements focusing on ?SynBioMs will likely also affect GMM which were not intended to be in the scope. This could be ?further clarified in the section.? ? Line 935: Please see our comments on ?extensively engineered? under the Glossary section below. ?	idem
127	Federal Office of Consumer protection and Food Safety (BVL), National Competent Authority	5.2. New hazards/risks	Lines 962-963: The potential hazard/risk posed to the gut microbiome is not specific for SynBioM products. The lines should therefore be deleted here.	These lines have been deleted.
128	FEFANA asbl	5.2. New hazards/risks	? Lines 951-953: This seems to be an unsubstantiated hypothesis rather than a validated risk for which a substantial scientific rationale and basis is available. If it is the former, the text should be deleted. If it is the latter, a proper reference should be provided, supporting this potential risk. [Same comment as in Section 3.3. above.]	Idem as response to response 67.
129	EuropaBio - The European Association for	5.2. New hazards/risks	Lines 951-953: This seems to be an unsubstantiated hypothesis rather than a validated risk for which a substantial scientific rationale and basis is available. If it is the former, the text should be deleted. If it is the latter, a	idem

	Bioindustries		proper reference should be provided, supporting this potential risk. [Same comment as in Section 3.3. above.]	
130	AMFEP	5.2. New hazards/risks	? Lines 951-953: This seems to be an unsubstantiated hypothesis rather than a validated risk for ?which a substantial scientific rationale and basis is available. If it is the former, the text should be ?deleted. If it is the latter, a proper reference should be provided, supporting this potential risk. ??[Same comment as in Section 3.3. above.]?	idem
131	Testbiotech	5.2. New hazards/risks	Any reference to new hazards and risks should also include paratransgenesis and SynBioM producing ncRNA, which can be relevant for a wide range of applications and should be explored in further case studies. In addition, the systemic risks due to the (intentional or unintentional) introduction of a large number of SynBioM with different traits and involving different species into the environment and the food and feed chain, which also inherit biological characteristics far beyond anything achieved by previous methods, also need to be addressed.	For non-coding RNA cases: see response to comment 40. See responses to comment 102 for holobiont and 47, 102, 110 for combinatorial effects.
132	POLLINIS	5.2. New hazards/risks	We think it is a good idea to include the concept of paratransgenesis (see 2.5 above) as it is a rapidly growing field including microorganisms and food-affected organisms (i.e. the honey bee).	Noted, idem as response to comments 40, 41 with the inclusion of additional cases.
133	ANSES	5.3. Adequacy of existing guidelines	The existing guidances and guidelines dealing with allergenicity remain adequate but some improvements and clarifications would be desirable (see next item).	Noted.

134	FEFANA asbl	5.3. Adequacy of existing guidelines	? Lines 968-970: The statement is important to clarify that, in practice, risk assessment is based on a product-based approach and not on a technique used to create the product. This will ensure proper enforcement of the respective regulations and also guarantee that the safety evaluation is based on the actual risks posed to consumers and the environment. ? Lines 1001-1006: This bullet point should be separated into two, i.e. ? The GMM guidance (EFSA GMO Panel, 2011) on the 90-day rodent studies (applicable primarily to Category 3 and 4 products) describes the importance of assessing the viability and the residence time of the GMM in the gut ecosystem. It also points out the need to study the interactions of the GMMs with the gut microbiota and their effects on digestive physiology and immune responses. ? Studies on the interactions with the gut microbiota and their effects on digestive physiology and immune responses would be required for Category 1 or 2 products only if such effects are to be expected. ? Line 1021: ?derived from? should be replaced by ?produced from?	Line 968: Sentences are considered sufficiently clear. Line 1001: Text adjusted. Line 1021: Text was adjusted.

135	EuropaBio - The European Association for Bioindustries	5.3. Adequacy of existing guidelines	Lines 968-970: The statement is important to clarify that, in practice, risk assessment is based on a product-based approach and not on a technique used to create the product. This will ensure proper enforcement of the respective regulations and also guarantee that the safety evaluation is based on the actual risks posed to consumers and the environment. Lines 1001-1006: This bullet point should be separated into two, i.e. ? The GMM guidance (EFSA GMO Panel, 2011) on the 90-day rodent studies (applicable primarily to Category 3 and 4 products) describes the importance of assessing the viability and the residence time of the GMM in the gut ecosystem. It also points out the need to study the interactions of the GMMs with the gut microbiota and their effects on digestive physiology and immune responses. ? Studies on the interactions with the gut microbiota and their effects on digestive physiology and immune responses would be required for Category 1 or 2 products only if such effects are to be expected. Line 1021: ?derived from? should be replaced by ?produced from?	idem

136	EFFCA - European Food and Feed Cultures Association	5.3. Adequacy of existing guidelines	EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? ? Lines 968-970: The statement is important to clarify that, in practice, risk assessment is based on ?a product-based approach and not on a technique used to create the product. This will ensure ?proper enforcement of the respective regulations and also guarantee that the safety evaluation is ?based on the actual risks posed to consumers and the environment.? ? Lines 1001-1006: This bullet point should be separated into two, i.e.? ??? The GMM guidance (EFSA GMO Panel, 2011) on the 90-day rodent studies (applicable ?primarily to Category 3 and 4 products) describes the importance of assessing the ?viability and the residence time of the GMM in the gut ecosystem. It also points out the ?need to study the interactions of the GMMs with the gut microbiota and their effects on ?digestive physiology and immune responses.? ??? Studies on the interactions with the gut microbiota and their effects on digestive ?physiology and immune responses would be required for Category 1 or 2 products only if ?such effects are to be expected.? ? Line 1021: ?derived from? should be replaced by ?produced from??	idem

137	AMFEP	5.3. Adequacy of existing guidelines	? Lines 968-970: The statement is important to clarify that, in practice, risk assessment is based on ?a product-based approach and not on a technique used to create the product. This will ensure ?proper enforcement of the respective regulations and also guarantee that the safety evaluation is ?based on the actual risks posed to consumers and the environment.? ? Lines 1001-1006: This bullet point should be separated into two, i.e.? ??? The GMM guidance (EFSA GMO Panel, 2011) on the 90-day rodent studies (applicable ?primarily to Category 3 and 4 products) describes the importance of assessing the ?viability and the residence time of the GMM in the gut ecosystem. It also points out the ?need to study the interactions of the GMMs with the gut microbiota and their effects on ?digestive physiology and immune responses.? ??? Studies on the interactions with the gut microbiota and their effects on digestive ?physiology and immune responses would be required for Category 1 or 2 products only if ?such effects are to be expected.? ? Line 1021: ?derived from? should be replaced by ?produced from??	idem
138	Federal Office of Consumer protection and Food Safety (BVL), National	5.4. Need for Updates of guidance or lack of methodologies	Lines 1032-1058: It can be observed that most updates considered necessary do not specifically refer to SynBioM products, but also to their non-GM and GM counterparts. Most SynBioM products can be treated as GMOs and do not need different regulations and guidances.	Noted.

	Competent Authority			
139	ANSES	5.4. Need for Updates of guidance or lack of methodologies	The existing guidances and guidelines dealing with allergenicity remain adequate but some improvements and clarifications would be desirable e.g. for evaluating the potential adjuvancity of proteins expressed in GMPs. In this respect, the search for identities with toxins needs to be improved by creating a toxin databank containing accurately selected proteins with confirmed adjuvant properties, that could be used to search for relevant identities. Another possible improvement should be made for evaluating the potential immunotoxic properties of peptides resulting from the proteolysis of proteins expressed by GMPs, by performing systematic docking of the suspected peptides to the basket of HLA-DQ2 and HLA-DQ8, using publicy available web docking servers.	Noted. Outside of the scope of this ToR as this opinion is not aimed at developing specific guidances. Limitations in the current guidances are reported in the opinion.
140	Evonik Operations GmbH	5.4. Need for Updates of guidance or lack of methodologies	Lines 1028-1031: In general, we very much welcome EFSA?s proposal to streamline the categorization of products. However, such categorization should be based on safety risks, we strongly recommend a slightly different categorization, as follows: ? Category 1: Products containing no live cells of the microorganism, nor DNA encoding sequences of concern ? Category 2: Products containing no live cells of the microorganism, but DNA encoding sequences of concern ? Category 3: Products consisting of or containing live cells of the microorganism Line 1087: This bullet point should start with: ?Only for	The comment is of interest but currently not followed for the categorisation, since not in the scope of the ToR (to check existing guidances). Text is adjusted.

			microorganisms containing sequences of concern: [?]?	
141	FEFANA asbl	5.4. Need for Updates of guidance or lack of methodologies	? Lines 1028-1031: We very much welcome EFSA?s proposal to streamline the categorization of products. ? However, since such categorization should be based on safety risks, we strongly recommend a slightly different categorization, as follows: ? Category 1: Products containing no live cells of the microorganism, nor DNA encoding sequences of concern and not containing XNA ? Category 2: Products containing no live cells of the microorganism, but DNA encoding sequences of concern and/or XNA ? Category 3: Products consisting of or containing live cells of the microorganism ? Lines 1051-1052 and 1067 - 1080: Please see our comments under sections 3.8 and 3.11 on gut microbiome. ? Lines 1054-1056: We appreciate the concerns with regard to the potential impact of living cell intake. This, however, should be closely related to the risk associated with the respective microorganism. Further, QPS strains should not be investigated in this way, as they are considered safe already. This should be clearly stated in this context. ? Lines 1064-1066: we fully support the conclusion expressed in this sentence. Therefore, and to avoid any doubts, the bullet point should be amended as follows: ?Therefore, potential risks first need to be substantiated and understood before suitable risk assessment tools can and shall be developed.? ? Line 1073: ?designed? should be replaced by ?explored?, to reflect the current status of science ? Line 1087: This bullet point	Idem as 140 for the categorisation. Other points of this comment were addressed as explained in previous responses: text was adjusted where considered adequate.

			should start with: ?Only for microorganisms containing sequences of concern: [?]?	
142	EuropaBio - The European Association for Bioindustries	5.4. Need for Updates of guidance or lack of methodologies	Lines 1028-1031: We very much welcome EFSA?s proposal to streamline the categorization of products. However, since such categorization should be based on safety risks, we strongly recommend a slightly different categorization, as follows: ? Category 1: Products containing no live cells of the microorganism, nor DNA encoding sequences of concern and not containing XNA ? Category 2: Products containing no live cells of the microorganism, but DNA encoding sequences of concern and/or XNA ? Category 3: Products consisting of or containing live cells of the microorganism Lines 1051-1052 and 1067 - 1080: Please see our comments under sections 3.8 and 3.11 on gut microbiome. Lines 1054-1056: We appreciate the concerns with regard to the potential impact of living cell intake. This, however, should be closely related to the risk associated with the respective microorganism. Further, QPS strains should not be investigated in this way, as they are considered safe already. This should be clearly stated in this context. Lines 1064-1066: we fully support the conclusion expressed in this sentence. Therefore, and to avoid any doubts, the bullet point should be amended as follows: ?Therefore, potential risks first need to be substantiated and understood before suitable risk assessment tools can and shall be developed.? Line 1073: ?designed? should be replaced by ?explored?, to reflect the current status of science Line 1087: This bullet point	idem

			should start with: ?Only for microorganisms containing sequences of concern: [?]?	
143	AMFEP	5.4. Need for Updates of guidance or lack of methodologies	? Lines 1028-1031: We very much welcome EFSA?s proposal to streamline the categorization of ?products.? ? IMPORTANT: However, since such categorization should be based on safety risks, we strongly ?recommend a slightly different categorization, as follows:? ??? Category 1: Products containing no live cells of the microorganism, nor DNA encoding ?sequences of concern and not containing XNA ??? Category 2: Products containing no live cells of the microorganism, but DNA encoding ?sequences of concern and/or XNA ??? Category 3: Products consisting of or containing live cells of the microorganism ? Lines 1051-1052 and 1067 - 1080: Please see our comments under sections 3.8 and 3.11 on gut ?microbiome. ? ? Lines 1054-1056: We appreciate the concerns with regard to the potential impact of living cell ?intake. This, however, should be closely related to the risk associated with the respective ?microorganism. Further, QPS strains should not be investigated in this way, as they are ?considered safe already. This should be clearly stated in this context.? ? Lines 1064-1066: we fully support the conclusion expressed in this sentence. Therefore, and to ?avoid any doubts, the bullet point should be amended as follows: ?Therefore, potential risks first ?need to be substantiated and understood before suitable risk assessment tools can and shall be ?developed.?? ? Line 1073: ?designed? should be replaced by ?explored?, to reflect the current	idem

			status of science ? Line 1087: This bullet point should start with: ?Only for microorganisms containing sequences of ?concern: [?]??	
144	Testbiotech	5.4. Need for Updates of guidance or lack of methodologies	There is need for further methodology and guidelines on: ? How to integrate scenarios that include potential impacts and interactions of accumulated and combinatorial effects caused by the (intended or unintended) presence of more than one SynBioM in a joint environment. ? How to apply effective cut-off criteria in the face of non-knowledge, a high degree of uncertainties and non-conclusive risk assessment. ? How to effectively limit and comprehensively control the release of SynBioM into the environment in regard to numbers, different traits and species; ? How to apply effective cut-off criteria in the face of non-knowledge, a high degree of uncertainties and non-conclusive risk assessment; ? How to detect and monitor the unintended presence of SynBioM and their DNA or XNA in the food chain and the environment.	For combinatorial effects and detection: see response to comment 47.
145	Federal Office of Consumer protection and Food Safety (BVL), National Competent Authority	6. Recommendations	Lines 1097-1099: It is welcomed that a strain-driven risk assessment approach instead of a technique-driven approach is recommended for SynBioM assessments.	Noted and covered in the opinion.

146	ANSES	6. Recommendations	Some recommendations for improvements of guidelines for allergenicity (see 5.4) should be useful to improve the evaluation of adjuvancity and immunotoxicity (non-IgE mediated allergenicity towards celiac diseased people). The existing guidances and guidelines dealing with allergenicity remain adequate but some recommendations for improvements of guidelines for allergenicity (see 5.4) should be useful to improve the evaluation of adjuvancity and immunotoxicity.	Text was adjusted for “needs for updates”. The comment is of interest but currently not followed, since not in the scope of the ToR (to check existing guidances).
147	Evonik Operations GmbH	6. Recommendations	Lines 1091-1093: We very much agree to further aim to ensure harmonized regulatory frameworks. Lines 1097-1098: We very much agree with a product-based approach for authorization rather than a process-based. This principle should be expanded to the entire EU GM regulatory framework.	Noted
148	FEFANA asbl	6. Recommendations	? Lines 1091-1093: We very much agree to further aim to ensure harmonized regulatory frameworks. ? Lines 1095-1096: This bullet point is highly misleading, as it implies that the scientific basis is sufficiently established to allow research on testing methods. To avoid confusion, it would be desirable to be much more explicit and clearer about what the next steps should or will be. First, exploratory research is required to discover whether omics tools have the potential to identify and/or monitor food safety risks more efficiently than other methods. And only once this has been established will it make sense to start research on testing methods. ? Lines 1097-1098: We very much agree with a product-based approach for	Text was adjusted.

			authorization rather than a process-based. This principle should be expanded to the entire EU GM regulatory framework.	
149	EuropaBio - The European Association for Bioindustries	6. Recommendations	Lines 1091-1093: We very much agree to further aim to ensure harmonized regulatory frameworks. Lines 1095-1096: This bullet point is highly misleading, as it implies that the scientific basis is sufficiently established to allow research on testing methods. To avoid confusion, it would be desirable to be much more explicit and clearer about what the next steps should or will be. First, exploratory research is required to discover whether omics tools have the potential to identify and/or monitor food safety risks more efficiently than other methods. And only once this has been established will it make sense to start research on testing methods. Lines 1097-1098: We very much agree with a product-based approach for authorization rather than a process-based. This principle should be expanded to the entire EU GM regulatory framework.	idem

150	EFFCA - European Food and Feed Cultures Association	6. Recommendations	EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? ? Lines 1091-1093: We very much agree to further aim to ensure harmonized regulatory ?frameworks.? ? Lines 1095-1096: This bullet point is highly misleading, as it implies that the scientific basis is ?sufficiently established to allow research on testing methods. To avoid confusion, it would be ?desirable to be much more explicit and clearer about what the next steps should or will be. First, ?exploratory research is required to discover whether omics tools have the potential to identify ?and/or monitor food safety risks more efficiently than other methods. And only once this has ?been established will it make sense to start research on testing methods.? ? Lines 1097-1098: We very much agree with a product-based approach for authorization rather ?than a process-based. This principle should be expanded to the entire EU GM regulatory ?framework.?	idem
151	AMFEP	6. Recommendations	? Lines 1091-1093: We very much agree to further aim to ensure harmonized regulatory ?frameworks.? ? Lines 1095-1096: This bullet point is highly misleading, as it implies that the scientific basis is ?sufficiently established to allow research on testing methods. To avoid confusion, it would be ?desirable to be much more explicit and clearer about what the next steps should or will be. First, ?exploratory research is required to discover whether omics tools have the potential to identify ?and/or monitor food safety risks more efficiently than other methods. And only once this has ?been established will it make sense to start	idem

			research on testing methods.? ? Lines 1097-1098: We very much agree with a product-based approach for authorization rather ?than a process-based. This principle should be expanded to the entire EU GM regulatory ?framework.?	
152	POLLINIS	6. Recommendations	We urge EFSA to include - The concept of holobiont ? must look at the risks not just for the microorganism but the organism as a whole and the context in which it lives - Develop technology assessment and horizon screening - Develop a better understanding of how to limit and understand the possibility of limitations of any release of microorganisms obtained by synthetic biology because of the possible unintended and intended risks - Respect and apply the precautionary principle	Idem response as to comment 102 (for holobiont) and 110 as well as Appendix B (for the precautionary principle).
153	FEFANA asbl	Abbreviations	? Delete PGRP ? abbreviation is not used in the text ? EFSA = European Food Safety Authority ? The following abbreviations used in the text are missing here: ? FF = Food & Feed ? GMP = Genetically Modified Plant (quite unfortunate use of this abbreviation, due to the more common use of this abbreviation for Good Manufacturing Practice) ? MC = Microbial Characterization or Molecular Characterization ? In the text of the document, abbreviations are often not introduced adequately, e.g. ? MC (line 43) ? GMP (line 79) ? FF (Table 2) ? TU (line 320) ? PMM (line 795) ? PPP (line 804)	The abbreviation list was corrected and extended.

154	EuropaBio - The European Association for Bioindustries	Abbreviations	Delete PGRP ? abbreviation is not used in the text EFSA = European Food Safety Authority The following abbreviations used in the text are missing here: ? FF = Food & Feed ? GMP = Genetically Modified Plant (quite unfortunate use of this abbreviation, due to the more common use of this abbreviation for Good Manufacturing Practice) ? MC = Microbial Characterization or Molecular Characterization ? In the text of the document, abbreviations are often not introduced adequately, e.g. ? MC (line 43) ? GMP (line 79) ? FF (Table 2) ? TU (line 320) ? PMM (line 795) ? PPP (line 804)	idem
155	EFFCA - European Food and Feed Cultures Association	Abbreviations	EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? ? EFSA = European Food Safety Authority ? The following abbreviations used in the text are missing here:? ??? FF = Food & Feed ??? GMP = Genetically Modified Plant (quite unfortunate use of this abbreviation, due to the ?more common use of this abbreviation for Good Manufacturing Practice)? ? In the text of the document, abbreviations are often not introduced adequately, e.g.? ??? MC (line 43)? ??? GMP (line 79)? ??? FF (Table 2)? ??? TU (line 320)? ??? PMM (line 795)? ??? PPP (line 804)?	idem

156	AMFEP	Abbreviations	? Delete PGRP ? abbreviation is not used in the text ? EFSA = European Food Safety Authority ? The following abbreviations used in the text are missing here:? ??? FF = Food & Feed ??? GMP = Genetically Modified Plant (quite unfortunate use of this abbreviation, due to the ?more common use of this abbreviation for Good Manufacturing Practice)? ??? MC = Microbial Characterization or Molecular Characterization ? In the text of the document, abbreviations are often not introduced adequately, e.g.? ??? MC (line 43)? ??? GMP (line 79)? ??? FF (Table 2)? ??? TU (line 320)? ??? PMM (line 795)? ??? PPP (line 804)?	idem
157	FEFANA asbl	Glossary	? Lines 1523-1525 (?extensively engineered?): Suggestion to delete. The authors state that they want to move away from the technique used to the characteristics of the product. "Extensively engineered" has no scientific basis, and in no way is a term or concept that supports the risk assessment. In this regard, please also amend the sentences in lines 119 and 935, accordingly. ? Line 1577: the correct term is Qualified Presumption of Safety ? In the text, in multiple locations, the term ?auxotrophic? is used erroneously, and should be replaced by ?autotrophic?: e.g. Table 5, Case 5; Table 6, Case 5; Table 11, Case 5 ? The phrases ?genes of concern? is used multiple times within the text (lines 408, 413, 478, 631) but it is not clearly defined what is included. In lines 477-478, virulence factors and toxins are named, in lines 631-633 ?harmful traits? are mentioned. It is important to clarify what is a gene of concern. For example, acquired AMR genes or antimicrobial	The term 'extensively engineered' was deleted from the opinion. Line 1577 was corrected. Auxotrophic has been replaced by autotrophic and the text was adjusted for case 5. The meaning of “genes of **potential** concern” is now more explained in the section 4.2 with the reference to existing EFSA document.

			resistance marker genes (ARM) would be a concern, while intrinsic AMR genes would not. However, in lines 913-915, this important differentiation is not made, suggesting any AMR gene would be of concern (?potentially harmful sequences such as antibiotic resistance genes?). This should be corrected accordingly.	
158	EuropaBio - The European Association for Bioindustries	Glossary	Lines 1523-1525 (?extensively engineered?): Suggestion to delete. The authors state that they want to move away from the technique used to the characteristics of the product. "Extensively engineered" has no scientific basis, and in no way is a term or concept that supports the risk assessment. In this regard, please also amend the sentences in lines 119 and 935, accordingly. Line 1577: the correct term is Qualified Presumption of Safety In the text, in multiple locations, the term ?auxotrophic? is used erroneously, and should be replaced by ?autotrophic?: e.g. Table 5, Case 5; Table 6, Case 5; Table 11, Case 5 The phrases ?genes of concern? is used multiple times within the text (lines 408, 413, 478, 631) but it is not clearly defined what is included. In lines 477-478, virulence factors and toxins are named, in lines 631-633 ?harmful traits? are mentioned. It is important to clarify what is a gene of concern. For example, acquired AMR genes or antimicrobial resistance marker genes (ARM) would be a concern, while intrinsic AMR genes would not. However, in lines 913-915, this important differentiation is not made, suggesting any AMR gene would be of concern (?potentially harmful	idem

			sequences such as antibiotic resistance genes?). This should be corrected accordingly.	
159	EFFCA - European Food and Feed Cultures Association	Glossary	EFFCA would like to support following points raised by AMFEP, FEFANA, EuropaBio:? ? Lines 1523-1525 (?extensively engineered?): Suggestion to delete. The authors state that they ?want to move away from the technique used to the characteristics of the product. "Extensively ?engineered" has no scientific basis, and in no way is a term or concept that supports the risk ?assessment.? ? Line 1577: the correct term is Qualified Presumption of Safety ? The phrases ?genes of concern? and is used multiple times within the text (lines 408, 413, 488, ??613) but it is not clearly defined what is included. In lines 477-478, virulence factors and toxins ?are named, in lines 631-633 ?harmful traits? are mentioned. It is important to clarify what is a ?gene of concern and what not. For example, acquired AMR genes or antimicrobial resistance ?marker genes (ARM) would be a concern, while intrinsic AMR genes would not. However, in lines ??913-915, this important differentiation is not made, suggesting any AMR gene would be of ?concern. This should be corrected accordingly. ? ? In the text, in multiple locations, the term ?auxotrophic? is used erroneously, and should be ?replaced by ?autotrophic?: e.g. Table 5, Case 5; Table 6, Case 5; Table 11, Case 5?	idem

160	AMFEP	Glossary	? Lines 1523-1525 (?extensively engineered?): Suggestion to delete. The authors state that they ?want to move away from the technique used to the characteristics of the product. "Extensively ?engineered" has no scientific basis, and in no way is a term or concept that supports the risk ?assessment. In this regard, please also amend the sentences in lines 119 and 935, accordingly. ? ? Line 1577: the correct term is Qualified Presumption of Safety ? In the text, in multiple locations, the term ?auxotrophic? is used erroneously, and should be ?replaced by ?autotrophic?: e.g. Table 5, Case 5; Table 6, Case 5; Table 11, Case 5 ? ? The phrases ?genes of concern? is used multiple times within the text (lines 408, 413, 478, 631) ?but it is not clearly defined what is included. In lines 477-478, virulence factors and toxins are ?named, in lines 631-633 ?harmful traits? are mentioned. It is important to clarify what is a gene ?of concern. For example, acquired AMR genes or antimicrobial resistance marker genes (ARM) ?would be a concern, while intrinsic AMR genes would not. However, in lines 913-915, this ?important differentiation is not made, suggesting any AMR gene would be of concern ??(?potentially harmful sequences such as antibiotic resistance genes?). This should be corrected ?accordingly. ?	idem

161	Testbiotech	References	In view of plans to introduce, without any kind of precedent, a large number of SynBioMs with different traits and from different species into the environment (and into the food and feed chain), which can 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. These efforts should take into account: ? Prospective technology assessment and horizon screening; ? Guidance on how to integrate scenarios that include potential impacts and interactions of accumulated and combinatorial effects caused by the presence of more than one SynBioM in a joint environment; ? The biological concept of the holobiont - taking into consideration that the risks of SynBioM cannot be assessed simply by looking at single cells in isolation. Without such a concept, any guidance for risk assessment will remain fragmentary; ? Guidance on how to effectively limit and comprehensively control the release of SynBioM into the environment in regard to numbers, different traits and species; ? Guidance on how to apply effective cut-off criteria in the face of non-knowledge, a high degree of uncertainties and non-conclusive risk assessment; ? Guidance which includes detection and methods to monitor the unintended presence of SynBioM and their DNA or XNA in the food chain and the environment; ? An evaluation of the QPS concept in regard to (i) unintended interactions within the cells as well as	Idem as responses to comments, 47, 110 and comment 102.

			(ii) new phenotypical characteristics of the cell populations within one species (such as growth of bacteria) and (iii) potential interactions, accumulated and combinatorial effects between organisms caused by the (intended or unintended) presence of more than one SynBioM in a specific environment.	
162	POLLINIS	References	1. EFSA Scientific Committee, et al., Scientific opinion on the evaluation of existing guidelines for their adequacy for the microbial characterisation and environmental risk assessment of microorganisms obtained through synthetic biology. EFSA Journal, 2020 18(10): p. 6263,. 2. Bellard, C., P. Cassey, and T. Blackburn, Alien species as a driver of recent extinctions. Biology Letters, 2016. 12(20150623). 3. Coutinho-Abreu, I., Z. Kun Yan, and M. Ramalho-Ortigao, Transgenesis and paratransgenesis to control insect-borne diseases: Current status and future challenges. Parasitol. Int. , 2010. 59(1): p. 1-8. 4. Rangberg, A., et al., Paratransgenesis: an approach to improve colony health and molecular insight in honey bees (Apis mellifera)? Integr Comp Biol, 2012. 52(1): p. 89-99. 5. Moran, N., J. Barrick, and S. Leonard, United States Patent Applicationm Publication: Engineered microbial population, in US 2019/0015528 A1. 2019. 6. Leonard, S., et al., Genetic engineering of bee gut microbiome bacteria with a toolkit for modular assembly of broad-host-range plasmids. ACS Synth Biol, 2018. 7(5): p. 1279?1290. 7. Leonard, S., et al., Engineered symbionts activate honey bee immunity and limit pathogens. Science, 2020.	Noted as part of Appendix B.

			367(6477): p. 573-576. 8. Borum, A., Microbiota and Its Importance in Honey Bees. Bee studies 2021. 13(1): p. 23-30. 9. Bonilla-Rossoa, G., et al., Honey bees harbor a diverse gut virome engaging in nested strain-level interactions with the microbiota Proceedings of the National Academy of Sciences, 2020. 117(13). 10. Kwong, W., et al., Dynamic microbiome evolution in social bees. Science Advances, 2017. 3(e1600513). 11. Rosenberg, E. and I. Zilber-Rosenberg, Microbes Drive Evolution of Animals and Plants: the Hologenome Concept. American society for microbiology, 2016. 7(2): p. e013595-15. 12. Xia, J., et al., Whitefly hijacks a plant detoxification gene that neutralizes plant toxins. Cell. 184(7): p. 1693-1705.	
163	JFDA			na

Abbreviations

AMFEP	Association of Manufacturers and Formulators of Enzyme Products
ANSES	French agency for Food, Environmental and Occupational Health & Safety
BVL	Federal Office of Consumer protection and Food Safety
EFFCA	European Food and Feed Cultures Association
FEFANA	EU Association of Specialty Feed Ingredients and their Mixtures
JFDA	Jordan Food and Drug Administration
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. Federal Office of Consumer Protection and Food Safety (BVL)

Chapter	Line Number	Comment
3.8. Gut microbiome and horizontal gene transfer		This chapter is more detailed and comprehensive, it gives a good oversight on the topic, however it differentiates from other chapters. For the sake of consistency and editorial uniformity all chapters should be kept similar in regard to background information, information on available guidances etc. EFSA: noted, some modifications for alignment were made but as this topic is under development in general, it requires more background information.
	543 to 545	This very valuable information together with corresponding information on available guidances/sections in other chapters should be readily summarized in a table and added to this document as an Annex EFSA: not followed as this is Opinion is not Guidance development.
	601 to 605	These are clearly medical applications and not food/feed

		EFSA decided to leave this information in the explanatory text section because products with these functions can also be food and feed.
	640 to 642	The sentence should be checked for completeness: "*An extensive risk assessment on the horizontal transfer of antimicrobial marker genes from GM plants to gut and environmental microbiota was (EFSA, 2009; presenting the joint work of the GMO and BIOHAZ Panels).*" Was what? EFSA: corrected
	Table 10	Editorial comment: the table should be editorially adjusted to other tables EFSA: checked
	Table 10, case 1	Redundancy: "no effect on the gut microbiome in every column" – please keep the text shorter EFSA: considered but kept for clarity
	Table 10, case 2	This conclusion is not comprehensible. Xeno amino acids or lantibiotics are "*constituents other than proteins"* The GMM-Guidance 2011, Section 2.4.1.2. (*Evaluation of constituents other than proteins)* states: *"New constituents other than proteins, as well as any anticipated changes in specific metabolic pathways due to the modification, should be evaluated. This may include toxicological testing on a case-by-case basis."* und *"If due to the modification of specific*

		metabolic pathways, the levels of naturally occurring metabolites have been changed an evaluation based on the knowledge of the physiological function and/or toxic properties of these constituents, as well as the anticipated changes in intake levels should be carried out. The result of this assessment would determine if, and to what extent, toxicological tests are required." This guidance should be considered as adequate. EFSA: text adjusted as " Guidances are not fully adequate" since the effect of the presence of xeno compounds may need special attention.
	667	Which methodologies are meant here? The requirements should be clearly stated to avoid misunderstandings EFSA: text adjusted
	669 to 671	It should be refrained from mixing of gaps in research/understanding and requirements for risk assessment. These lines emphasize that the knowledge on gut microbiome and its interactions is limited so far due to its complexity and more studies are desirable. However, a strict distinction should be made between a demand for fundamental research and RA requirements. It also applies to "omics" studies, which are an excellent choice for studies of complex communities, however

		are not suitable for RA due to the lack of standardisation. EFSA: text adjusted

B.2. POLLINIS

General comment:

We feel that EFSA has not complied with this binding precautionary principle. EFSA should ensure all the needed guidelines are developed before any release, as the role of EFSA is to ensure that risks are fully evaluated. Those applications of synthetic biology to micro-organisms, including yeast, viruses, protists, bacteria, fungi account for the majority of life on earth and form the basis of life and connections to all existing species. Microorganisms have the possibility to put into danger or change existing relationships within the biosphere. Moreover, the rise of zoonotic diseases raises more concern to the already complicated relationships between microorganisms, plants and other organisms, including humans. We urge EFSA to apply the precautionary principle on applications of synthetic biology to microorganisms.

EFSA response: Guidance documents for risk assessment are made publicly available as a guide for applicants before they make their safety dossiers. Therefore, guidance documents are always "generic" to provide a risk assessment approach and to anticipate as much as possible the specific data requirements needed for certain types of products. Before any release, EFSA will evaluate risks on a case by case basis when a dossier for market authorization comes in. Such risks will be evaluated in line with the intended use of the microorganism and include the' potential impact on human and animal health and the environment.

Footnotes:

1. EFSA Scientific Committee, et al., *Scientific opinion on the evaluation of existing guidelines for their adequacy for the microbial characterisation and environmental risk assessment of microorganisms obtained through synthetic biology.* EFSA Journal, 2020 **18**(10): p. 6263,.
2. Bellard, C., P. Cassey, and T. Blackburn, *Alien species as a driver of recent extinctions.* Biology Letters, 2016. **12**(20150623).
3. Coutinho-Abreu, I., Z. Kun Yan, and M. Ramalho-Ortigao, *Transgenesis and paratransgenesis to control insect-borne diseases: Current status and future challenges.* Parasitol. Int. , 2010. **59**(1): p. 1-8.
4. Rangberg, A., et al., *Paratransgenesis: an approach to improve colony health and molecular insight in honey bees (Apis mellifera)?* Integr Comp Biol, 2012. **52**(1): p. 89-99.
5. Moran, N., J. Barrick, and S. Leonard, *United States Patent Applicationm Publication: Engineered microbial population*, in *US 2019/0015528 A1*. 2019.

6. Leonard, S., et al., *Genetic engineering of bee gut microbiome bacteria with a toolkit for modular assembly of broad-host-range plasmids.* ACS Synth Biol, 2018. **7**(5): p. 1279–1290.
7. Leonard, S., et al., *Engineered symbionts activate honey bee immunity and limit pathogens.* Science, 2020. **367**(6477): p. 573-576.
8. Borum, A., *Microbiota and Its Importance in Honey Bees.* Bee studies 2021. **13**(1): p. 23-30.
9. Bonilla-Rossoa, G., et al., *Honey bees harbor a diverse gut virome engaging in nested strain-level interactions with the microbiota* Proceedings of the National Academy of Sciences, 2020. **117**(13).
10. Kwong, W., et al., *Dynamic microbiome evolution in social bees.* Science Advances, 2017. **3**(e1600513).
11. Rosenberg, E. and I. Zilber-Rosenberg, *Microbes Drive Evolution of Animals and Plants: the Hologenome Concept.* American society for microbiology, 2016. **7**(2): p. e013595-15.
12. Xia, J., et al., *Whitefly hijacks a plant detoxification gene that neutralizes plant toxins.* Cell. **184**(7): p. 1693-1705.