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## Evaluation of existing guidelines for their adequacy for the molecular characterisation and environmental risk assessment of genetically modified plants obtained through synthetic biology

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### Abstract

Synthetic Biology (SynBio) is an interdisciplinary field at the interface of engineering and biology aiming to develop new biological systems and impart new functions to living cells. EFSA has been asked by the European Commission to evaluate SynBio developments in agri-food with the aim of identifying the adequacy of existing guidelines for risk assessment and determine if updated guidance is needed. The scope of this opinion covers the molecular characterisation and environmental risk assessment of such genetically modified plants obtained through SynBio, meant to be for cultivation or food and feed purposes. The previous work on SynBio by the non-food scientific Committees (2014, 2015) was used and complemented with the output of a horizon scanning exercise, which was commissioned by the EFSA to identify the most realistic and forthcoming SynBio cases of relevance to this remit. The horizon scan did not identify other sectors/advances in addition to the six SynBio categories previously identified by the non-food scientific committees of the European Commission. The exercise did show that plant SynBio products reaching the market in the near future (next decade) are likely to apply SynBio approaches to their development using existing genetic modification and genome editing technologies. In addition, three hypothetical SynBio case studies were selected by the working group of the Panel on Genetically Modified Organisms (GMO), to further support the scoping exercise of this Scientific Opinion. Using the selected cases, the GMO Panel concludes that the requirements of the EU regulatory framework and existing EFSA guidelines are adequate for the risk assessment of SynBio products to be developed in the next 10 years, although specific requirements may not apply to all products. The GMO Panel acknowledges that as SynBio developments evolve, a need may exist to adjust the guidelines to ensure they are adequate and sufficient.

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## Summary

Synthetic Biology (SynBio) is an interdisciplinary field at the interface of engineering and biology aiming to develop new biological systems and impart new functions to living cells with potential applications in the food and feed system that would require a prior authorisation in Europe.

This Opinion addresses four requests by the European Commission as described in the terms of reference on the safety evaluation of SynBio developments in agri-food uses:

- 1) identification of newer sectors/advances in the agri-food sector considered among SynBio developments (excluding bioremediation, de-extinction, bioweapons/biopreparedness, medical use, biofuels);
- 2) identification of potential risks and potential novel hazards SynBio could pose for the environment (restricted to wildlife and excluding humans and farmed animals);
- 3) evaluating the adequacy of existing guidelines for risk assessment of current and near future SynBio products (arriving to EU market in the next decade);
- 4) identification of specific areas where updated guidance is needed.

The scope of this opinion covers genetically modified (GM) plants expected to be deliberately released into the environment, including cultivation. This focuses on the 2 out of the 6 previously reported SynBio categories: (1) genetic part libraries and methods, and (2) DNA synthesis and genome editing, as defined by the European Commission's non-food Scientific Committees.<sup>1</sup> The other four categories such as minimal cells and protocells, xenobiology and citizen science were excluded from this work package. The previous work on SynBio<sup>1</sup> was used as basis for this work and was complemented by the output of a horizon scanning, which was commissioned by EFSA to identify the SynBio developments in the agri-food sectors likely to enter the market in the next decade. The GMO Panel reviewed the results of the horizon scanning and the available published information on SynBio developments and selected three hypothetical case studies with the potential to reach the market within the next 10 years:

Case study 1: a GM sweet maize engineered to produce vitamin B12 by the transgenic insertion of a single molecular stack containing multiple engineered genes from the B12 biosynthesis pathway, not normally present in plants;

Case study 2: a low-gluten GM wheat produced by targeted mutations of multiple  $\alpha$ -gliadin genes using CRISPR/Cas9 genome editing, and,

Case study 3: a fungal-resistant oilseed rape obtained by transgenic insertion of an existing plant resistance gene engineered to recognise a broader range of pathogens as well as the deletion of additional genes for pathogen susceptibility using genome editing.

These case studies were used to assess the adequacy and applicability of the requirements as described in the Commission Implementing Regulation 503/2013<sup>2</sup> and the EFSA Guidance on the environmental risk assessment of genetically modified plants.<sup>3</sup>

The GMO Panel came to the following conclusions based on the three hypothetical case studies plus the outcome of the horizon scanning exercise:

- 1) no other sectors/advances among the six SynBio categories which were already identified by the non-food Scientific Committees, were found.
- 2) no potential novel hazards compared to established techniques of genetic modification and no novel potential risks in terms of impact on humans, animals and the environment, were identified.
- 3) the evaluation of existing guidelines using the three selected case studies, showed that the current requirements are adequate and sufficient for the risk assessment of such cases although not always applicable to all the three selected case studies.
- 4) For SynBio developments in the wider future (> 10 years), a need may exist to adjust the requirements of the guidelines to ensure that they are adequate and sufficient for plants engineered with SynBio. In particular cases, other risk assessment approaches may be needed

<sup>1</sup> SCENIHR, SCCS and SCHER, 2014. Synthetic Biology I Definition, Opinion, September 2014. Available online: [http://ec.europa.eu/health/scientific\\_committees/emerging/docs/scenihr\\_o\\_044.pdf](http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_044.pdf) SCENIHR, SCCS and SCHER (2015) Synthetic Biology II – Risk assessment methodologies and safety aspects, Opinion, May 2015. Available online: [http://ec.europa.eu/health/scientific\\_committees/emerging/docs/scenihr\\_o\\_048.pdf](http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_048.pdf) SCENIHR, SCCS and SCHER, 2015. Synthetic Biology III – Research priorities, Opinion, December 2015. Available online: [http://ec.europa.eu/health/scientific\\_committees/emerging/docs/scenihr\\_o\\_050.pdf](http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_050.pdf)

<sup>2</sup> <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013R0503&from=EN>

<sup>3</sup> <https://www.efsa.europa.eu/en/efsajournal/pub/1879>

that do not rely on history of safe use and the current comparative approach. Modelling of the characteristics of engineered plants, including their expected behaviour in different environments might aid and improve the comparative analysis and ERA of SynBio genetically modified plants (GMPs) in a given receiving environment.

## Table of contents

Abstract.....	1
Summary.....	3
1. Introduction.....	6
1.1. Definitions for SynBio for the Terms of Reference .....	7
1.2. Background and Terms of Reference as provided by the requestor.....	7
1.3. Interpretation of the Terms of Reference and Scope .....	8
2. Data and methodologies.....	9
2.1. Existing guidelines and ad hoc expert Working Group .....	9
2.2. Horizon scanning of SynBio developments.....	9
2.3. Selection of case studies.....	10
2.4. Consultation.....	11
3. Assessment.....	12
3.1. Evaluation of Commission Implementing Regulation 503/2013 on the adequacy of the requirements for the molecular characterisation of genetically modified plants .....	12
3.1.1. Information related to the genetic modification (design, methodologies) .....	12
3.1.2. Characterisation of the modified/inserted/deleted sequences .....	12
3.1.3. Information on the expression of the inserted/modified sequences (incl. protein expression) .....	13
3.1.4. Genetic stability of the inserted/modified sequences and phenotypic stability of the GMP.....	13
3.1.5. Bioinformatic analyses .....	14
3.1.6. Interactions in stacks.....	14
3.1.7. MC Conclusions and Outlook .....	14
3.2. Evaluation of the EFSA guidance (EFSA GMO Panel, 2010) on the adequacy of the requirements for the environmental risk assessment of genetically modified plants.....	15
3.2.1. Cross-cutting considerations.....	15
3.2.2. Persistence and invasiveness including plant-to-plant gene flow .....	15
3.2.3. Plant to microorganisms gene transfer .....	15
3.2.4. Interactions of the GM plant with target organisms.....	16
3.2.5. Interactions of the GM plant with non-target organisms.....	16
3.2.6. Impact of the specific cultivation, management and harvesting techniques.....	16
3.2.7. Effects of biogeochemical processes .....	16
3.2.8. Effects on human and animal health.....	16
3.2.9. Overall risk evaluation and conclusions.....	17
3.2.10. Post-market environmental monitoring plan.....	17
3.2.11. ERA and PMEM Conclusions and Outlook.....	17
4. Overall Conclusions and Recommendations .....	18
References.....	19
Abbreviations.....	20

## 1. Introduction

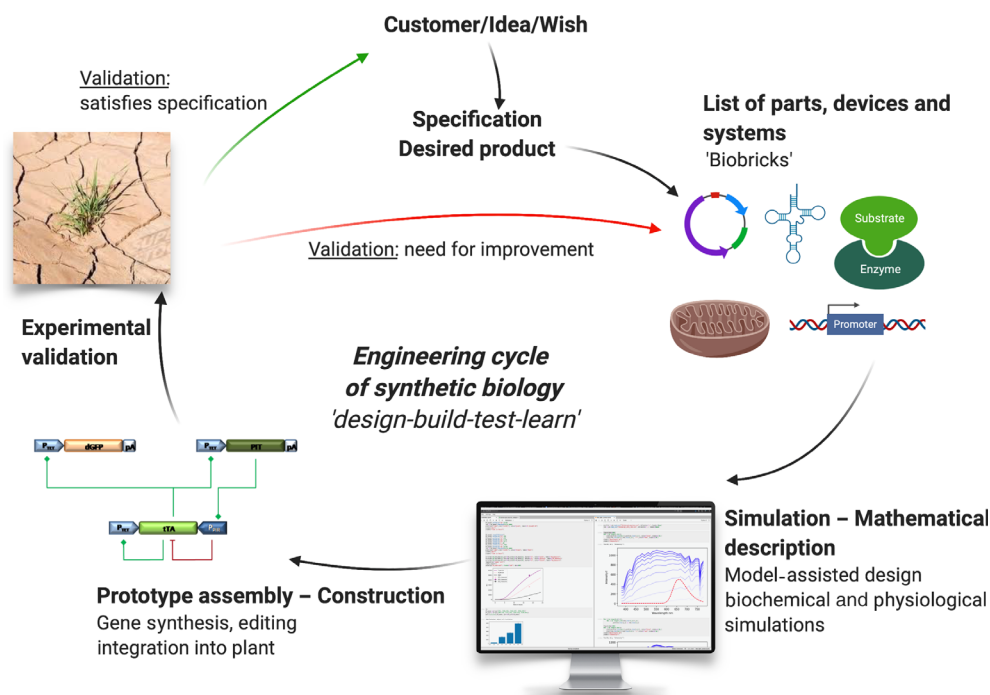
SynBio is an interdisciplinary field at the interface of engineering and biology aiming to develop new biological systems and impart new functions to living cells. It employs engineering principles such as standardisation, modularity, modelling and computer-aided design to improve the predictability of the bioengineering process. Standardisation and modularity speed up the engineering process by allowing parts to be easily exchanged and iterative engineering cycles of 'design-build-test-learn' to improve the functionality. At the same time, the application of modelling and computer-aided design informs and predicts the outcomes of different engineering strategies and, subsequently, improves the design by including quantitative data generated from the 'design-build-test-learn' cycles (Figure 1). By combining engineering, life sciences and computational modelling, SynBio is expanding the range of applications and products that are being developed.

SynBio has potential applications in the food and feed chain that would under current legislation require a pre-market authorisation in Europe. Some of those applications may include the deliberate release of such genetically modified organisms in the environment (e.g. SynBio plants or SynBio microorganisms for plant growth promotion or plant protection) and hence will be subject to an environmental risk assessment (ERA). This is also reported by the Scientific Advice Mechanism (SAM) explanatory note of April 2017 on new techniques in agricultural biotechnology,<sup>4</sup> outlining the agricultural application of new techniques in the fields of SynBio and gene drive. Previously, in 2014 and 2015, the European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Consumer Safety (SCCS) published three opinions (SCENIHR, SCCS, and SCHER, 2014, 2015a, 2015b) on SynBio,<sup>1</sup> addressing six SynBio developments: (1) genetic part libraries and methods; (2) minimal cells and designer chassis; (3) protocells and artificial cells; (4) xenobiology; (5) DNA synthesis and genome editing; and (6) citizen science (Do-It-Yourself biology). The opinions addressed the definition of SynBio, risk assessment methodologies and safety aspects, risks to the environment and biodiversity and research priorities in the field of SynBio. These non-food Scientific Committees concluded in their opinions that new SynBio applications may be assessed using current risk assessment methodology for genetically modified organisms (GMOs) and that the rapidly evolving SynBio technologies may require existing methodologies to be revisited at regular intervals and improved when necessary to continue ensuring their safety.

Therefore, as a proactive measure, the European Commission requested the European Food Safety Authority (EFSA) for an opinion on GMOs developed using SynBio approaches and the implications, if any, for risk assessment methodologies. EFSA identified a total of six opinions to be developed, according to organism group and risk assessment aspects.

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<sup>4</sup> [https://ec.europa.eu/info/publications/new-techniques-agricultural-biotechnology\\_en](https://ec.europa.eu/info/publications/new-techniques-agricultural-biotechnology_en)



**Figure 1:** Synthetic biology is a new discipline bridging engineering with life sciences. It applies basic engineering principles for the rational modular, combinatorial assembly of biological parts into higher order complex signaling and metabolic structures with novel, desired functionalities. Key to the strategy is the implementation of the engineering cycle in biology relying in mathematical modeling for the design and quantitative functional characterization of the molecular parts and for guiding the assembly, implementation, and optimization of the individual modules and networks. A prototype is assembled and experimentally tested. If the product satisfies the specifications a priori established, then it is considered finished. If not, the product reenters the cycle for improvement, the process continuing until it fulfils the specifications

### 1.1. Definitions for SynBio for the Terms of Reference

SynBio has been previously defined as follows by the non-food Scientific Committees upon request of the European Commission<sup>5,6</sup>: 'Synthetic biology is the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms'. This definition is used as a starting point for the present opinion due to the request of the European Commission to build on the Opinions of the non-food Scientific Committees.

The Convention on Biological Diversity<sup>7</sup> further clarified that 'While there is no internationally agreed definition of "synthetic biology", key features of synthetic biology include the "de novo" synthesis of genetic material and an engineering-based approach to develop components, organisms and products'.

This further clarification establishes the link for the request to support the European Union (EU) in the work under the Convention on Biological Diversity and the Cartagena Protocol on Biosafety.<sup>8</sup>

<sup>5</sup> <https://ec.europa.eu/research/sam/index.cfm?pg=agribiotechnology>

<sup>6</sup> SCENIHR, SCCS, SCHER (2014) Synthetic Biology I Definition, Opinion, September 2014. Available online: [http://ec.europa.eu/health/scientific\\_committees/emerging/docs/scenihr\\_o\\_044.pdf](http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_044.pdf)

<sup>7</sup> The Convention on Biological Diversity is a multilateral treaty under the auspices of the United Nations Environment Program. Its major goals are the conservation of biodiversity, sustainable use of the components of biodiversity, and fair and equitable sharing of benefits arising from genetic resources stemming from biodiversity See: <https://www.cbd.int/doc/meetings/cop/cop-12/information/cop-12-inf-11-en.pdf>

<sup>8</sup> The Cartagena Protocol on Biosafety to the Convention on Biological Diversity was adopted on 29 January 2000, and entered into force on 11 September 2003. The Cartagena Protocol presently has 171 contracting parties, excluding large LMO exporters such as Argentina, Canada and the United States. See: <https://www.cbd.int/doc/legal/cartagena-protocol-en.pdf>

## 1.2. Background and Terms of Reference as provided by the requestor

Building on the non-food Scientific Committees opinions and taking into account available literature and previous analyses carried out by EU Member States and at international level, the Commission asked EFSA,<sup>9</sup> in accordance with Art 29 (1) of Regulation (EC) No 178/2002, for an opinion on GMOs developed through SynBio and their implications for risk assessment methodologies. The scope of the present mandate is limited to agri-food uses.<sup>10</sup> In this context:

- 1) EFSA was asked to consider whether and which **newer sectors/advances** should be considered among SynBio developments, in addition to the six identified by the Scientific Committees (ToR1).
- 2) EFSA was requested to identify, where possible, **potential risks** in terms of impact on humans, animals and the environment that current and near future SynBio developments could pose; in this respect EFSA is also asked **to identify potential novel hazards** compared to established techniques of genetic modification<sup>11</sup> (ToR2).
- 3) EFSA was requested to determine whether the **existing guidelines** for risk assessment are adequate and sufficient for current and near future SynBio developments or whether there is a need for updated guidance (ToR3).
- 4) In the latter case EFSA was requested to identify the specific areas where such **updated guidance** is needed (ToR4).

EFSA is also requested to provide technical and scientific expertise on the risk assessment of GMOs obtained through SynBio to support the EU in the work under the Convention on Biological Diversity and the Cartagena Protocol on Biosafety.

## 1.3. Interpretation of the Terms of Reference and Scope

The mandate received from the European Commission was split into six work packages (WP) by EFSA to be reflected in six Opinions to be developed:

- 1) Microbial characterisation and ERA of genetically modified microorganisms (GMMs) (WP1)
- 2) Molecular characterisation and ERA of genetically modified plants (GMPs) (WP2)
- 3) Food and feed risk assessment of GMMs (WP3)
- 4) Food and feed risk assessment of GMPs (WP4)
- 5) Molecular characterisation and ERA of genetically modified animals (WP5)
- 6) Food and feed risk assessment of genetically modified animals (WP6)

The current Opinion is addressing WP2.

The scope of this plant-specific opinion considers two out of the six SynBio categories previously identified by the non-food Scientific Committees, namely genetic part libraries and methods, and DNA synthesis and genome editing; therefore excluding minimal cells and designer chassis, and protocells and artificial cells by definition, xenobiology and citizen science. In addition, developments regarding targeted mutations are addressed in the EFSA GMO Panel opinion on 'Applicability of the EFSA Opinion on site-directed nucleases type 3 for the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide-directed mutagenesis'.<sup>12</sup>

The following interpretations to the terms of references (ToR) are made for the development of this Opinion, in agreement with the European Commission<sup>9</sup>:

- Not all of the six developments previously identified by the non-food Scientific Committees were considered relevant.
- 'Near future': for this mandate this is interpreted as products with the potential to reach the EU market in the next decade. This is reflected in Section 2.3 when selecting hypothetical case studies.

<sup>9</sup> See correspondence under mandate M-2018-0205 in the EFSA register of questions: <http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?3>

<sup>10</sup> For the purpose of this mandate agri-food uses means agri/food/feed products falling within the remit of EFSA.

<sup>11</sup> For the purpose of this mandate the terms 'established techniques of genetic modification' refers to various genetic engineering techniques that have been significantly used over the last 30 years to produce genetically modified organisms, such as those that have been authorised under Directive 2001/18/EC and Regulation (EU) No 1829/2003.

<sup>12</sup> <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2020.6299/full>



- 'Agri-food uses': On footnote 5 of the mandate '*For the purpose of this mandate agri-food uses means agri/food/feed products falling within the remit of EFSA*', further clarifications were needed to determine which applications fall within the remit of EFSA and this mandate, and the available time frame. The limited time frame available to complete this Opinion led to the explicit exclusion of bio-remediation applications from this mandate. The following uses are also excluded from this mandate: de-extinction, bio-weapons/bio-preparedness, medical use, and biofuels (see Section 2.2 and the EFSA External Scientific Report<sup>13</sup>).
- For the purpose of this opinion, ToR2 was limited to deliberate release into the environment only (including wildlife). The exposure to humans and farmed animals (accidental or deliberate) will be specifically addressed in WP4, covering the food/feed aspects of GM plants obtained through SynBio approaches (SynBio GMP).
- 'Existing guidelines for risk assessment': see Section 2.1

The opinion is produced not only to support the European Commission, but is also meant for the public, scientific community and stakeholders, companies and institutions interested in or dealing with safety of SynBio plant developments.

## 2. Data and methodologies

### 2.1. Existing guidelines and ad hoc expert Working Group

EFSA established an ad hoc expert Working Group of the GMO Panel on the molecular characterisation (MC) and ERA of GMPs obtained by SynBio (hereafter referred to as SynBio GMPs) that met regularly to address the mandate of the European Commission.<sup>14</sup> In delivering its Scientific Opinion, the GMO Panel, together with the *ad hoc* expert Working Group, considered the current GMO legislation and corresponding EFSA guidance documents. The documents that are relevant for MC and ERA of SynBio GMPs for deliberate release into the environment are presented in Table 1.

The GMO Panel notes that EFSA has published more recent guidance on the agronomic and phenotypic characterisation of GMPs (EFSA GMO Panel, 2015) relevant for the comparative assessment of GMPs obtained through SynBio as well as guidance on post-market environmental monitoring (PMEM) that clarifies the objectives, tasks, tools and requirements for PMEM (EFSA GMO Panel, 2011b), also relevant for the PMEM of GMPs obtained through SynBio.

**Table 1:** Existing guidelines and regulatory framework for MC and ERA

Reference	Title
Directive 2001/18/EC	Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms
Commission Directive (EU) 2018/350	Commission Directive (EU) 2018/350 of 8 March 2018 amending Directive 2001/18/EC of the European Parliament and of the Council as regards the environmental risk assessment of genetically modified organisms
EC Regulation No 503/2013	Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006
EFSA GMO Panel (2011a)	Guidance for risk assessment of food and feed from genetically modified plants
EFSA GMO Panel (2010)	Guidance on the environmental risk assessment of genetically modified plants

In the first instance, the Working Group (WG) reviewed the results of the horizon scanning on SynBio developments in plants, as relevant to ToRs 1 and 2 (Section 2.2), and the available published information (up to March 2019) on this topic. On the basis of this review, three hypothetical case studies were selected (Section 2.3) to assess the adequacy of the requirements per section outlined in the Commission Implementing Regulation 503/2013 (CIR 503/2013) and the Guidance on the ERA of GMPs (EFSA GMO Panel, 2010) which serve as the reference documents for the assessment of the

<sup>13</sup> <https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2020.en-1687>

<sup>14</sup> <https://www.efsa.europa.eu/sites/default/files/wgs/gmo/wg-plant-synbio-era.pdf>

adequacy of existing requirements for MC and ERA (with focus on cultivation) of GMPs, respectively, for the selected case studies. This assessment is reported in Section 3 and fulfils ToRs 2, 3 and 4.

## 2.2. Horizon scanning of SynBio developments

ToR1 (and part of ToR2) is addressed with a horizon scanning.

Synthetic biology is an evolving research field with the potential for the development of novel techniques and approaches for the design and development of GMOs. In order to get an overview of the SynBio developments for plants in the agri-food sector that are likely to enter the market in the next decade, EFSA requested a contractor via a procurement call, to perform a horizon scanning exercise. The information extracted from the review of the full text publications is presented in Appendix A of the EFSA External report.<sup>13</sup>

Plants developed using SynBio approaches were identified using a search strategy including a systematic literature review, expert interviews and a collation of companies applying SynBio to plants.<sup>15</sup> This revealed that current projects are aiming to optimise oil composition in plants (Napier et al., 2014), confer nitrogen fixation to non-legumes (Rogers and Oldroyd, 2014), improve photosynthetic capacity (Kubis and Bar-Even, 2019), improve abiotic stress tolerance to salt, drought or heat stress or a combination (Cabello et al., 2014). However, only few of these projects are sufficiently advanced so that they can be considered as likely to lead to market releases in the near future. The expert consultations that were part of this horizon scanning confirmed that most SynBio developments in plants are far from application, although the search found a number of companies that support/pursue the development of SynBio products.

The horizon scanning highlights that GM plant products reaching the market in the next 10 years are likely to result from existing technologies including those resulting to the insertion of transgenes and genome editing. However, the WG considered that for some of these plants there is a difference in the applied approaches, namely that SynBio strategies such as modelling and analytical approaches to improve the predictability of the engineering are applied during the development process thereby enabling more complex traits.

## 2.3. Selection of case studies

SynBio approaches are typically *not* being applied to achieve simple gain-of-function traits encoded by a single gene such as herbicide tolerance or pest resistance. In contrast, SynBio approaches are being applied to engineer complex, quantitative traits controlled by multiple genes (e.g. photosynthetic capacity and nutrient use efficiency); for the design of traits that require lengthy multigene pathways (e.g. to produce new metabolites); and for the *de novo* design of proteins able to perform new or expanded functions.

A shortlist was made (by the WG) of plants being engineered using SynBio approaches based on existing literature and state of the art, with the potential to reach the market within the next 10 years (Table 2).

**Table 2:** Survey of existing SynBio developments by the WG experts

	Description of the new trait	Technological complexity	Current status	Example reference
1	Plants or plant cell cultures producing ingredients for food, e.g. colour	Low	Proof of concept	Appelhagen et al. (2018)
2	Plant food or feed engineered for increased nutrition – carotenoids, e.g. astaxanthin, PUFAs (polyunsaturated fatty acids), vitamins, etc.	Low to medium	Feeding trials	1) Breitenbach et al. (2016) 2) Betancor et al. (2016)
3	Deletion of undesirable gene products from crop plants (food or feed), e.g. allergenic, gluten, bitterness, etc.	Low to medium	Proof of concept	Sánchez-León et al. (2018)

<sup>15</sup> <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2020.EN-1687>

	Description of the new trait	Technological complexity	Current status	Example reference
4	Plants engineered to produce disease-prevention agents, e.g. virus-like particles, via oral delivery (animal feed)	Medium to high	Proof-of-concept to product development	1. Kolotilin et al. (2014) 2. Lemire et al. (2018) 3. <a href="http://www.frontlinegenomics.com/news/15211/antibiotic-resistant-bacteria-tackled-new-drug-using-crispr">http://www.frontlinegenomics.com/news/15211/antibiotic-resistant-bacteria-tackled-new-drug-using-crispr</a>
5	Plants engineered to improve complex traits e.g. yield, photosynthetic capacity, tolerance to biotic and abiotic-stress	Medium to high	Proof-of-concept; field-testing in model plant species	1. South et al. (2019) 2. De la Concepcion et al. (2019)

Based on known advances identified in the horizon scanning exercises, the following hypothetical case studies were outlined by experts in the field with a view to representing products likely to be developed using state-of-the art SynBio approaches with the potential to reach the market within the next 10 years:

- Case Study 1:** GM sweet maize engineered to produce vitamin B12 (cobalamin) not normally synthesised by plants, by the transgenic insertion of a molecular stack containing multiple engineered genes from one or more bacterial vitamin B12 biosynthesis pathways. There are currently no reports of plants engineered to produce B12, however, the 25 genes required to make B12 have been identified and the pathway has been reconstituted in non-B12-producing bacteria (Ko et al., 2014). Projects investigating bioenrichment of plants are already in progress (e.g. <https://gtr.ukri.org/projects?ref=BB%2FS014020%2F1>). The ability to build and deliver multi-gene constructs to plants has been widely demonstrated. However, obtaining proper function of a complex pathway in a new organism requires tight and differential regulation of each of the enzymes in the pathway as well as the potential engineering of each enzyme for optimal function in a plant cell. Reconstructing this large pathway in a plant is a highly complex task likely to integrate multiple SynBio approaches including computational modelling of multiple variants of pathway components to select the most promising iterations; optimising the expression level of each gene including the use of conditional switches to maintain the desired levels and patterns of expression within the plant; metabolic modelling of the cell to identify endogenous genes that prevent the accumulation of the end product.
- Case Study 2:** Non-transgenic, gluten-free wheat. A low-gluten wheat was reported in 2018 obtained by the introduction of targeted mutations of multiple  $\alpha$ -gliadin genes using CRISPR/Cas9 genome editing (Sánchez-León et al., 2018). In this case, the mutations were achieved using a transgene and lines that lacked the transgene and only contained the desired mutations were subsequently obtained by segregation after self-pollination. While numerous published studies have demonstrated that it is possible to segregate transgenes in the next generation in this manner (Chen et al., 2019), other studies have demonstrated that it is possible to produce wheat plants with multiple targeted mutations without integration of any transgene and by direct delivery of a protein-RNA complex (e.g. Liang et al., 2017). In case study 2, we anticipate a targeted approach to remove gluten achieved without the introduction of a transgene and all target genes to be successfully and precisely edited resulting in a gluten-free wheat. While plants with a small number of mutations have already reached the market, the large number of mutations required to achieve gluten-free wheat is far beyond any plant previously assessed. This is likely to require SynBio approaches to correctly identify all gliadins and glutenins in the hexaploid genome of bread wheat and to identify an engineering strategy that introduced mutations of the correct nature and positions in each gene to prevent the accumulation of any peptide fragments associated with initiation of the inflammatory cascade.
- Case Study 3:** Fungus-resistant GM oilseed rape obtained by transgenic insertion of a plant resistance gene and genome engineering of susceptibility genes. This case study is based on the combination of two reported studies: first, on a plant engineered to recognise a broader

range of pathogens by structure-guided engineering of a nucleotide binding-leucine-rich repeat (NLR) protein (De la Concepcion et al., 2019). In plants, NLR proteins recognise proteins secreted by pathogens resulting in an immune response that leads to plant cell death at the point of infection and, therefore, resistance. Second, on the deletion of additional genes for pathogen susceptibility using genome engineering tools such as CRISPR/Cas9 (Zaidi et al., 2018). The combination of strategies will result in plants resistant to a broader range of fungal pathogens. To achieve this requires SynBio approaches including model-guided redesign of the protein from its crystal structure to identify which mutations will enable the identification of new pathogens. It will then require the insertion of this redesigned protein into a plant in which endogenous susceptibility genes have been previously identified and edited or deleted.

These specific case studies were selected because, even though they have been produced using existing GM technologies including those resulting to the insertion of transgenes and genome editing (and therefore resemble current GMPs) their complexity is likely to require the application of SynBio approaches, as described in the Introduction (Section 1).

## 2.4. Consultation

In line with its policy on openness and transparency, EFSA consulted EU Member States and its stakeholders via an online public consultation. Between April and May 2020, interested persons were invited to submit their comments on the draft GMO Panel Scientific Opinion.<sup>16</sup> Following this consultation process, the document was revised by the GMO Panel and the experts of its ad hoc expert Working Group.

The outcome of the online public consultation is published on the EFSA's website.<sup>17</sup>

## 3. Assessment

### 3.1. Evaluation of Commission Implementing Regulation 503/2013 on the adequacy of the requirements for the MC of genetically modified plants

#### 3.1.1. Information related to the genetic modification (design, methodologies)

Sufficient information on the genetic modification is required for risk assessment, as part of the MC of GMPs, including the description of methods used, the nature and source of vector used, and the source of and function of nucleic acids intended for insertion (Sections 1.2.1.1–1.2.1.3 of CIR 503/2013).

The description of the methods used for the genetic modification in each of the three case studies can be provided according to the Section 1.2.1.1 of CIR 503/2013.

In case of a transgene being introduced, the current requirements of this section on the nature and source of vectors used (Section 1.2.1.2) and the characterisation of the donor organism, nucleotide sequences intended to be inserted, and information for all insertion sites (Section 1.2.1.3 of CIR 503/2013), remain adequate and applicable.

However, the SynBio methods used for the genetic modification of plants may differ from those of existing GM plants on which CIR 503/2013 is based and the information to be provided may therefore be different (e.g. CRISPR/Cas9 genome editing). For example, in case studies 2 and 3, genome editing approaches are used, where Cas9 activity can be provided either from a co-delivered mRNA or the protein itself. The level of details should be enough to allow EFSA: a) to identify the modifications potentially generated (deletion or nucleotide changes) and b) to characterise the sequences actually modified in the plant.

Therefore, the considerations provided in Sections 1.2.1.1–1.2.1.3 of CIR 503/2013, are adequate and sufficient for the three case studies, although specific requirements may not be needed or may need to be adapted, depending on the SynBio methods used.

#### 3.1.2. Characterisation of the modified/inserted/deleted sequences

The description of the trait(s) and characteristics introduced or modified (Section 1.2.2.1 of CIR 503/2013) and the information on the sequences actually inserted or deleted (Sections 1.2.2.2 of CIR 503/2013), are required for risk assessment.

<sup>16</sup> Published at <http://www.efsa.europa.eu/en/calls/consultations>

<sup>17</sup> <http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2021.EN-2000/full>

The general description of traits and characteristics of the GMPs can be provided in all three case studies following the CIR 503/2013.

The information on the sequences inserted/deleted allows for the molecular description of the event. It consists of the description of the insertion(s) of the intended sequence, as well as the unintended insertion of other sequences in the genome in case transgenes are used. This information is relevant to case studies 1 and 3 and can be obtained following the CIR 503/2013.

In case of modification of endogenous sequences by gene editing (case study 2 and partially case study 3), the characterisation of the intended sequence modification can be achieved by sequencing the target locus. In general, site-directed nucleases (SDNs) will introduce DNA breaks at the target locus with high specificity, resulting in mutations at this locus. However, SDNs may also cut the DNA at sequences showing extensive sequence similarity with the target locus and located elsewhere in the genome, resulting in off-target mutations. The EFSA GMO Panel has addressed the safety relevance of these SDN-related potential off-target mutations in two different opinions.<sup>12,18</sup> The GMO Panel concluded that as the mutations at DNA breaks are the result of the activity of the endogenous repair machinery, the off-target changes introduced by genome editing will be similar to those that occur after the repair of naturally occurring DNA breaks and also similar, but far fewer, than those that occur after using other established mutagenesis techniques that also introduce DNA breaks (e.g. radiation mutagenesis). Therefore, taking into account all of the above, the GMO Panel considers that the analysis of potential off-targets on a regular basis would be of very limited value for the risk analysis. Moreover, although, several bioinformatic tools are available for off-target prediction (e.g. Cas-OFFinder; Bae et al., 2014), the intraspecies and intravarietal variability would not always allow for a reliable prediction of potential off-target mutations.

Identification and risk assessment of potential new open reading frames (ORFs) in case studies 1 and part of 3, can be carried out the same way as for traditional applications, following the requirements and recommendations laid down in CIR 503/2013 and EFSA guidance (EFSA GMO Panel, 2011a). For the endogenous gene-edited genes in case studies 2 and 3, these requirements cannot be directly applied, since the concepts and definition of junction site, flanking region and event, would need to be reconsidered.

Therefore, the considerations provided in Sections 1.2.2.1 and 1.2.2.2 of the CIR 503/2013 are adequate and sufficient for the three case studies, although some requirements may not be needed or may need to be adapted depending on the SynBio methods used.

### 3.1.3. Information on the expression of the inserted/modified sequences (incl. protein expression)

In Section 1.2.2 of the CIR 503/2013, information on the expression of inserts (Section 1.2.2.3) is required, regarding whether the intended inserted/modified sequences result in intended changes at the protein, RNA and/or metabolites level. The study of potential unintended expression of ORFs (identified under Section 1.2.2.2, discussed here in Section 3.1.2) that would raise safety concerns is also required.

In case study 1, the introduced pathway enzymes (from about fifteen genes) required to produce the target metabolite (vitamin B12) should be characterised and the expression levels should be determined. In this case, it means the characterisation of about 15 proteins, but in future applications that aim to produce more complex metabolites or several different metabolites, this could increase to 100 or more proteins. Although, in principle the same techniques can be used as for measuring one protein, in practice it will be challenging to perform protein characterisation as currently done, for numerous newly expressed proteins.

In case study 2, the protein levels of the  $\alpha$ -gliadins would need to be assessed.

In case study 3, the expression level of both inserted and modified engineered proteins can be assessed as is done currently.

Therefore, the considerations provided in Section 1.2.2.3 of the CIR 503/2013 are adequate and sufficient and are thus applicable to the three case studies used for the adequacy assessment of existing guidelines.

<sup>18</sup> <https://www.efsa.europa.eu/en/efsajournal/pub/2943>

### 3.1.4. Genetic stability of the inserted/modified sequences and phenotypic stability of the GMP

Section 1.2.2.4 of CIR 503/2013 requires the demonstration of the genetic and phenotypic stability of the genetic modification. It contains requirements for the demonstration of the stability of transgenes over several generations that would be directly applicable to case study 1 and part of the genetic modifications of case study 3. When the genetic modification is achieved by genome editing, e.g. case study 2 and some of the genetic modifications of case study 3, demonstrating the genetic stability would involve determining that the nucleotide changes introduced by gene editing are stable over several generations, by sequencing the modified region, and that the introduced trait(s) are stable.

In case of stacked events, the considerations of Section 1.2.2.4 of CIR 503/2013 are also applicable.

Therefore, the considerations provided in Section 1.2.2.4 of CIR 503/2013 are adequate and sufficient for the three case studies, but cannot be directly applied when GMPs are obtained by gene editing because the CIR 503/2013 requires demonstration of the stability of transgenes. In these cases, assessing genetic stability is still relevant and would consist of demonstrating that the nucleotide change(s) and introduced trait(s) are stable.

### 3.1.5. Bioinformatic analyses

In Sections 1.2.2.2 and 1.2.2.5 of CIR 503/2013, bioinformatics analysis shall be conducted as part of the characterisation of the sequences actually inserted or modified and of the expression of these sequences. This includes the characterisation of new ORFs created as a consequence of the genetic modification. All new ORFs as defined in the CIR 503/2013 and EFSA guidance (EFSA GMO Panel, 2011a) shall be assessed for similarities to allergens and toxins. Depending on the information gathered under the MC and the bioinformatic predictions, further risk assessment may be needed. This part of the adequacy assessment of existing guidelines will be under the remit of the food and feed risk assessment, which will be addressed by the fourth work package as listed in Section 1.3.

In addition, the probability of horizontal gene transfer (HGT) when transgenes have been inserted should be assessed by bioinformatic tools in case studies 1 and 3, according to Section 1.2.2.5 of CIR 503/2013 and the EFSA Explanatory Note to the guidance on DNA sequence similarity searches using bioinformatic analyses to identify GM plant sequences with sufficient identity to promote homologous recombination and impact on horizontal gene transfer from plants to microorganisms (EFSA, 2017). The potential associated risks are addressed by the ERA (see Section 3.2.3).

Therefore, the considerations provided in Sections 1.2.2.2 and 1.2.2.5 of CIR 503/2013, are adequate and sufficient, but not applicable for all three case studies.

### 3.1.6. Interactions in stacks

The considerations on stacks provided in Section 1.2.2.3 of CIR 503/2013 are adequate and sufficient, when GM plants obtained by SynBio are combined by conventional crossing.

### 3.1.7. MC Conclusions and Outlook

The GMO Panel assessed the regulatory framework (CIR 503/2013) and existing guideline (EFSA GMO Panel, 2011a) on MC (see Section 2.1) and found that the requirements would be adequate and sufficient for the risk assessment of SynBio GMPs, although specific requirements cannot be directly applied to all the three case studies and may therefore not be needed or may need to be adapted depending on the SynBio methods used.

The MC of GMPs as currently performed will be sufficient to conclude on the structure and expression of the inserted/modified sequences and on their genetic and phenotypic stability, as required. Also, as discussed in the different MC sections, some specific requirements referring to the introduced transgenes, would not be relevant or may need to be adapted for gene edited sequences.

A general aspect related to the concepts used in some parts of the existing guidelines is that the technologies currently adopted as SynBio, are often based on altering the plant genome using gene editing instead of introducing a transgene. Therefore, in order to cover all technologies used, the term *modification* rather than *transformation* is more suited in the assessment of MC requirements while the concepts of event, junction site and flanking region may need to be reconsidered.

In addition, one potential difference between traditional applications and future SynBio applications is the scale of the changes introduced. Although the number of sequences intended for modification is expected to be far greater in SynBio GMPs, this does not necessarily imply that novel methods of assessment should be applied to characterise the genetic modifications themselves. However, as noted in Section 2.3, SynBio approaches can be used to engineer quantitative traits controlled by multiple genes, to design multigene pathways, and for the *de novo* design of genes able to perform new or expanded functions. Based on the state of the art for SynBio, a large increase in the complexity and diversity of the new traits is expected in future SynBio applications compared to traditional applications. Therefore, additional approaches and new technologies may be needed to risk assess the SynBio plants.

Moreover, SynBio approaches may require the introduction/modification of a high number of sequences that may not be genetically linked, similarly to what happens in GM plants with stacked events. In case of stacked events, the CIR 503/2013 requires determining that each of the transformation event stacked in the plant has the same molecular properties and characteristics as in the plant with the single transformation event. The current practice has been to finalise the risk assessment of each of the single event before assessing the stack, to be able to compare the stack alongside with each of the single (or sub-stacks) already assessed. This approach may be challenging or even unfeasible for certain SynBio plants with a high number of inserted/modified sequences.

### **3.2. Evaluation of the EFSA guidance (EFSA GMO Panel, 2010) on the adequacy of the requirements for the environmental risk assessment of genetically modified plants**

The considerations provided in Section 1 (Introduction) and Sections 2.1 and 2.2 (Strategies for ERA of GM plants) of the EFSA guidance (EFSA GMO Panel, 2010) are adequate for the three case studies.

#### **3.2.1. Cross-cutting considerations**

The considerations provided in Sections 2.3.1–2.3.4 of the EFSA guidance (EFSA GMO Panel, 2010) are adequate for the three case studies, as well as the considerations provided in Section 2.3.5 of EFSA GMO Panel (2010) in the case that the SynBio plants would be combined by conventional crosses.

#### **3.2.2. Persistence and invasiveness including plant-to-plant gene flow**

The GMO Panel made the following considerations for the adequacy assessment:

- Case studies 1 and 2: The intended traits, i.e. increased vitamin B12 content and reduced  $\alpha$ -gliadin content, respectively, are not meant to alter the plant's reproductive biology and life cycle characteristics. The considerations provided in Section 3.1 of EFSA (EFSA GMO Panel, 2010) related to the assessment of potential unintended effects caused by the genetic modification, however, remain applicable for these two cases.
- Case study 3: The intended trait (increased resistance to fungal plant pathogens) is not meant to alter the plant's reproductive biology and life cycle characteristics. However, in the presence of high levels of disease pressure the potential exists for SynBio GM oilseed rape plants to transiently persist within a production site and/or the wider environment. Both the considerations related to the trait and unintended effects provided in Section 3.1 of EFSA (EFSA GMO Panel, 2010) remain relevant to consider for this case study.

In conclusion, all three case studies should be considered through problem formulation as outlined in Section 3.1 of EFSA (EFSA GMO Panel, 2010), whose considerations are adequate.

#### **3.2.3. Plant to microorganisms gene transfer**

The GMO Panel made the following considerations for the adequacy assessment:

- Case study 1: Genes were integrated in the plant genomic DNA through transgenesis. Given that the make-up of the first case study resembles that of GMPs assessed so far by the GMO Panel, the approach and data requirements described in Section 3.2 of EFSA EFSA GMO Panel, 2010) remain applicable;

- Case study 2: As described in Section 2.3, no exogenous DNA has been introduced into the genome of the low-gluten wheat. Therefore, the data requirements described in Section 3.2 of EFSA (EFSA GMO Panel, 2010) are no longer considered relevant to assess the potential for HGT to microorganisms and the environmental consequences;
- Case study 3: The fungal-resistant GM oilseed rape has been obtained through transgenesis and genome editing. In the case of transgenesis, the same observations as for case study 1 apply, and in the case of genome editing, the same observations as for case study 2 apply.

The GMO Panel considers that if no exogenous DNA of sufficient length (see EFSA, 2017) has been inserted in the SynBio GMP (as may be the case for genome editing), only information on the genetic modification (under MC, Section 3.1.1) and characterisation of the modified/inserted/deleted genes (under MC, Section 3.1.2) would be needed in order to decide whether the potential for HGT from GMPs to microorganisms and its environmental consequences needs to be assessed. In case of genome editing, where there is no exogenous DNA of sufficient length inserted that allows homologous recombination, HGT assessment is not applicable.

In conclusion, the considerations provided in Section 3.2 of EFSA (EFSA GMO Panel, 2010) are adequate, but not necessarily applicable for all of the three case studies.

#### 3.2.4. Interactions of the GM plant with target organisms

The GMO Panel made the following considerations for the adequacy assessment:

- Case studies 1 and 2: The intended traits, i.e. increased vitamin B12 content and reduced  $\alpha$ -gliadin content, respectively, are not designed to confer resistance to certain plant pests or pathogens. Therefore, the considerations provided in Section 3.3 of EFSA (EFSA GMO Panel, 2010) are not relevant to these case studies;
- Case study 3: The intended trait in this oilseed rape is designed to confer resistance to fungal plant pathogens. Based on natural evolutionary processes, this could lead to the emergence of fungal strains virulent against the modified host plant. Therefore, the considerations provided in Section 3.3 of EFSA (EFSA GMO Panel, 2010) on data collection for characterising the potential of resistance development of target organisms and their exposure to the GMP, remain applicable for this case study.

In conclusion, the considerations provided in Section 3.3 of EFSA (EFSA GMO Panel, 2010) are adequate, but not applicable for all of the three case studies.

#### 3.2.5. Interactions of the GM plant with non-target organisms

The GMO Panel made the following considerations for the adequacy assessment:

- Case studies 1 and 2: The intended trait is not designed to control any plant pest or pathogen. Therefore, only the considerations provided in Section 3.4 of EFSA (EFSA GMO Panel, 2010) related to the assessment of potential unintended effects caused by the genetic modification remain applicable for these two cases.
- Case study 3: The newly introduced/modified NLR leads to a disease-resistant phenotype. Therefore, all considerations provided in Section 3.4 of EFSA (EFSA GMO Panel, 2010) for assessing impacts on non-target organisms remain applicable.

The testing of the toxicity potential of every single newly expressed proteins, including their potential for synergism, under laboratory conditions will entail practical challenges if an entire new metabolic pathway is introduced and a suite of newly expressed proteins may require evaluation.

In conclusion, all three case studies should be considered through problem formulation as outlined in Section 3.4 of EFSA (EFSA GMO Panel, 2010), whose considerations are adequate for the three case studies.

#### 3.2.6. Impact of the specific cultivation, management and harvesting techniques

Like any other conventional GMPs, SynBio plants may lead to changes in management practices, either directly linked to the specific trait(s) introduced (e.g. insect resistance or insecticide use) or due to induced changes commonly observed in agriculture (soil tillage, nitrogen application or crop rotation). The three case studies are no exception. Although only case study 3 is likely to lead to direct changes in crop management (altered application of fungicides), all three may lead to new



management systems and should be considered through problem formulation as outlined in Section 3.5 of EFSA (EFSA GMO Panel, 2010), whose considerations are adequate for the three case studies.

### 3.2.7. Effects of biogeochemical processes

Like other conventional GMPs, SynBio plants may lead to changes in biogeochemical processes, either directly linked to the specific trait(s) introduced or due to changes in management practices (see Section 3.2.5). The three case studies are no exception. Although case study 3 is more likely to lead to changes in biogeochemical processes (e.g. due to altered application of fungicides), all three may lead to changes in biogeochemical processes and should be considered through problem formulation as outlined in Section 3.6 of EFSA (EFSA GMO Panel, 2010), whose considerations are adequate for the three case studies.

### 3.2.8. Effects on human and animal health

The GMO Panel made the following considerations for the adequacy assessment:

- Case studies 1, 2 and 3: Since these three case studies are intended for food/feed uses, it is assumed that a food/feed risk assessment has been conducted. Hence, the accidental intake and exposure to plant material via contact or inhalation of pollen or dust from processed plants would be covered and does not need to be addressed in the ERA.

The considerations provided in Section 3.7 of EFSA (EFSA GMO Panel, 2010) were evaluated and found adequate for the three case studies.

In cases where the SynBio plant is developed for non-food and feed applications, the ERA will be based on the assumption that the risk assessment of a possible accidental intake will be conducted in the frame of the food/feed risk assessment, in line with the Commission Directive 2001/18/EC and CIR 503/2013.

### 3.2.9. Overall risk evaluation and conclusions

The considerations provided in Section 3.8 of EFSA (EFSA GMO Panel 2010) are adequate for the three studies. The case-by-case and problem formulation approach are considered sufficient to establish which data are relevant for ERA.

### 3.2.10. Post-market environmental monitoring plan

The considerations provided in Section 4 of EFSA (EFSA GMO Panel, 2010) and the most up to date guidance for PMEM provided in EFSA GMO Panel (2011b) are adequate for the three case studies.

### 3.2.11. ERA and PMEM Conclusions and Outlook

The GMO Panel assessed the existing guideline (EFSA GMO Panel 2010) and regulatory framework (Commission Directive (EU) 2018/350) on ERA (see Section 2.1) and found that while not all aspects of the guidance are necessarily relevant for all of the three case studies, for those studies where the guidance is relevant it is both adequate and sufficient.

However, for future SynBio plants resulting phenotypes may be more complex than those considered in the selected case studies (e.g. altered plant composition and nutritional value, improved photosynthesis, increased tolerance/resistance to abiotic and biotic stress, better nitrogen fixation, altered plant–microbiome interactions), therefore challenging current risk assessment approaches. More specifically, it is important to highlight the following:

SynBio approaches may affect various metabolic pathways, lead to numerous changes in the agronomic/phenotypic and compositional characteristics of the GMP, and result in altered interactions with the plant's receiving environments, challenging the comparative safety assessment as currently prescribed by EFSA GMO Panel (2010, 2011a). Indeed, actual impacts of SynBio GMPs with complex traits affecting various metabolic pathways are likely to be more context-dependent (in terms of genetic background, receiving environments and management practices);

Consequently, for SynBio GMPs in which a complex trait such as photosynthesis has been engineered, or in which multiple novel traits have been conferred, the following points should be considered:

- 1) the selection of relevant endpoints to test for their agronomic/phenotypic characterisation may need to be adapted on a case-by-case basis (see also EFSA GMO Panel, 2015);
- 2) the concept of comparator may have to evolve, as finding a suitable comparator with a genetic background as close as possible to the GMP, may entail challenges (e.g. traits conferring tolerance to abiotic stresses as the near-isogenic counterpart may be susceptible to these stresses)
- 3) the implementation and interpretation of the statistical tests of comparison with a non-GM comparator and equivalence with non-GM reference varieties (EFSA GMO Panel, 2015) may have to evolve considering the likely difficulties to set clear boundaries between the intended phenotype, associated pleiotropic effects and other traits of the plants and
- 4) more emphasis may have to be put on investigating potential interactions related to the management of generated SynBio GMPs across the environments in which they could be cultivated. This may be done by considering different genetic backgrounds across various receiving environments but this would entail challenges in the practical implementation and analysis. Therefore, a more predictive approach that models the behaviour of a given construct in a specific background and in a given receiving environment might be considered by taking advantage of the advancement of approaches in ecosystem modelling.

SynBio employs tools and approaches from computing and engineering, such as modelling and computer-aided design, in order to inform and predict the outcomes of different engineering strategies. These tools and approaches used in the engineering phase may aid the comparative analysis and ERA of SynBio GMPs in a given receiving environment.

Based on the state of the art for SynBio, current approaches of testing the ecotoxicity potential of every single newly introduced protein will no longer be feasible.

Future SynBio GMPs may also trigger a discussion on the strengths and limitations of PMEM, due to their complexity and the potential number of metabolic pathways affected, the actual behaviour of Synbio plants and their environmental impacts are likely to be more context-dependent than current GMPs. Therefore, predicting their impacts in all receiving environments during the pre-market risk assessment will entail challenges. There may be a need to consider whether possible additional uncertainties related to potential adverse environmental effects of a SynBio GMP could be assessed through PMEM.

In this context, problem formulation remains key to frame the ERA of future SynBio GMP applications following the case-by-case approach, thereby ensuring that existing knowledge is organised and used efficiently for their ERA and consider new predictive approaches, methods and tools.

#### **4. Overall Conclusions and Recommendations**

The GMO Panel assessed the requirements in the regulatory framework (CIR 503/2013) and the existing guidelines (EFSA GMO Panel 2011a) for the MC of GMPs, and the Commission Directive (EU) 2018/350) and existing guidance (EFSA GMO Panel, 2010) for the environmental risk assessment of GMPs, and concluded on the adequacy and applicability of the current methodologies for the risk assessment of SynBio plants that are likely to be marketed in the coming years based on the horizon scanning and the selected case studies. While these three case studies helped to focus on the current status of plant SynBio applications, SynBio developments are rapidly evolving and therefore the chosen cases may not be representative for all potential future applications.

In response to the specific ToRs of the mandate received by EFSA:

- 1) the GMO Panel did not identify other sectors/advances among the six SynBio categories identified by the non-food Scientific Committees, following a literature search-based horizon scanning exercise of plant SynBio developments in the agri-food sector;
- 2) based on the adequacy assessment of existing guidelines, no novel potential risks in terms of impact of SynBio GMPs on humans, animals and the environment, and no novel hazards compared to established techniques of genetic modification, were identified for the three case studies used. This is also supported by the outcome of the literature search-based horizon scanning exercise which highlights that most plant SynBio products reaching the market in the next 10 years are likely to result from existing technologies including the insertion of transgenes and genome editing and therefore resemble current GMPs. However, SynBio

developments are constantly evolving and therefore the current cases may not be representative for all future applications.

- 3) the evaluation of requirements in the existing guidelines, assessed using the selected case studies, led to the conclusion that the current risk assessment requirements and methodologies are adequate and sufficient for the risk assessment of such cases, although some of these requirements are not always applicable to all the three selected case studies. The GMO Panel acknowledges that as SynBio developments evolve, a need may exist to adjust the guidelines to ensure they are adequate and sufficient.
- 4) The areas recommended for updating for the RA of SynBio plants, may include:
  - the specific molecular data requirements for introduced transgenes, as they are not directly applicable when the modification is obtained using only gene editing approaches; because of the use of gene editing techniques, the terminology used in the current guidelines does not always apply; 'genetic modification' may be more suited than 'genetic transformation';
  - the concepts of event (single and stack) for SynBio plants;
  - the approaches for characterising and assessing complex traits;
  - the selection of relevant agronomic/phenotypic characterisation endpoints on a case-by-case basis;
  - the concept of comparator, as finding a suitable comparator with a genetic background as close as the SynBio GMP, may entail challenges in more complex future SynBio cases.
  - Other risk assessment approaches that do not rely on the current comparative approach may need to be considered. Modelling of the characteristics for engineered organisms, including their expected behaviour in different environments might aid and improve the comparative analysis and ERA of SynBio GMPs in a given receiving environment;
  - the 'design' factor in the SynBio GMP offers the possibility to address safety issues already identified during the *design-build-test-learn* cycle of the SynBio approach ('safe by design');
  - more emphasis may be given on the possible interactions between the SynBio GMP genotypes, their management and the receiving environments in which they are to be cultivated, by exploiting the advancement of approaches in ecosystem modelling;
  - need to consider whether possible additional uncertainties related to potential adverse environmental effects of a SynBio GMP could be better assessed through PMEM.

Future applications are difficult to predict as technical barriers still need to be overcome. However, forthcoming applications may include plants that, produce novel (designed, rather than transferred from another species) metabolites, express *de novo* designed enzymes with novel functions, have been engineered with signalling networks to better tolerate and adapt to changes in their environmental conditions, have an expanded recognition of pathogens, are engineered to optimise the uptake, assimilation and use of nutrients, have improved photosynthetic activity, and plants with synthetic organelles that perform novel functions.

Additional expertise may be needed to risk assess the increase in the diversity of the new traits expected in future SynBio applications compared to traditional applications, based on the state of the art for SynBio. Additional areas for potential updating may be identified for food/feed risk assessment which would determine the adequacy assessment of existing guidelines in food/feed safety. This will be addressed by the fourth work package (WP4) as listed in Section 1.3 of this opinion.

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## Abbreviations

CIR	Commission Implementing Regulation
ERA	environmental risk assessment
GMP	genetically modified plants
GMO	genetically modified organisms
HGT	horizontal gene transfer
ORF	open reading frames
PMEM	post-market environmental monitoring
RA	risk assessment
SC	Scientific Committee
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SDN	site-directed nucleases
SynBio	Synthetic Biology
SAM	Scientific Advice Mechanism
ToR	Terms of Reference
WG	Working Group
WP	work package