Update from EFSA’s Synthetic Biology WG

Nikoletta Papadopoulou
Scientific Officer
In 2012 EC commissioned 3 Scientific committees to deliver 3 opinions on Synthetic Biology:

- Scientific committee on Emerging and Newly identified Health Risks (SCENIHR)
- Scientific committee on Health and Environmental Risks (SCHER)
- Scientific committee on Consumer Safety (SCCS)

These opinions addressed:

- Definition of Synthetic biology
- Risk assessment methodologies and safety aspects
- Risks to the environment and biodiversity
- Research priorities in the field of SynBio
The Scientific Committees (SCs) defined synthetic biology as “the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms”. 
Scientific committees concluded that new SynBio developments may be assessed using current GMO RA methodologies. However, rapidly evolving technologies may require existing methodologies to be revisited at regular intervals and improved when necessary.

In 2017 the Scientific Advice Mechanism (SAM) published an explanatory note which included an outline of possible agricultural applications of new techniques in the fields of Synbio.

6 SynBio developments were assessed:
- Genetic part libraries and methods
- Minimal cells and designer chassis
- Protocells and artificial cells
- Xenobiology
- DNA synthesis and genome editing
- Citizen science (Do-it-yourself biology)

These technologies, methods and principles enable for faster and easier design and manufacturing of GMOs.
EC asked EFSA for an opinion on GMOs developed through synthetic biology and their implications for risk assessment methodologies. The scope is limited to agri-food uses and requests EFSA:

- To reflect whether and which newer sectors/advances should be considered among SynBio developments, in addition to the 6 identified;

- To identify, where possible, potential risks in terms of impact on humans, animals and the environment for current or near future SynBio developments. Identify novel hazards as compared to established GMO techniques;

- 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;

- In case guidances need to be updated, to identify the specific areas where such updated is needed.
ToR Interpretations for the development of this opinion:
- deliberate release into the environment
- reaching the market in the next decade
- exclusion of bioremediation applications (also de-extinction, bioweapons/biopreparedness, medical use, biofuels)

Overarching mandate, encompasses different EFSA units (Agri/food uses);

All relevant EFSA guidance documents for at least 6 SynBio developments for three organism groups (plants, animals, micro-organisms) will be considered;

It was agreed with the EC to split up the mandate in different work packages.
Reference documents

Regulation
- Directive 2001/18/EC
- Implementing Regulation (EU) 503/2013
- Directive (EU) 2018/350

Guidance
- 2010 EFSA GMO Panel GD for ERA of GMPs
- 2011 EFSA GMO Panel GD for ERA of GMMs
- 2011 EFSA GMO Panel GD for RA of FF from GMPs
- 2018 EFSA FEEDAP Panel GD for the characterisation of microorganisms used as feed additives or as production organisms
Two focused multidisciplinary EFSA working groups were established:

- **Plant SynBio ERA**, chaired by Ewen Mullins
- **GMM SynBio ERA**, chaired by Pier Sandro Cocconcelli

Two EFSA procurement contracts reporting the results of literature searches for SynBio developments:

- **GMM**: RIVM (NL), cecile.van.der.vlugt@rivm.nl
- **Plant SynBio ERA**: JKI (DE), ralf.wilhelm@julius-kuehn.de

Assessment of GD based on SynBio case studies, for applications likely to reach the market in 10 yrs; different complexity levels
Selection of case studies: inclusion criteria

- Products consisting or containing viable genetically modified organisms, as defined in Directive 2001/18 therefore able to replicate or transfer genetic material;
- GMMs covered include Archaea, bacteria, Eukarya;
- Products related to “agri-food uses meaning agri/food/feed products falling within the remit of EFSA”;
- Products deliberately released into the environment for experimental, scaling up or commercial reasons
- Product possibly reaching the EU market during the next decade.
EFSA procurement contract NP/EFSA/SCER/2018/03 to Institute for Biosafety in Plant Biotechnology (SB), Julius Kühn-Institut - Federal Research Centre for Cultivated Plants, Germany;

- Literature search for plant SynBio developments in the agri-food sector;

- Final report delivered 30 April 2019 authored by: Katharina Unkel, Doerthe Krause, Thorben Sprink, Frank Hartung, Ralf Wilhelm;

- SynBio in plants is currently less advanced than in microorganisms
Metabolic engineered plants will enter into market in the next 10 years (import to EU)

Continuous process of improving (stepwise) mainly less complex modifications (e.g. fatty acid composition) in already existing crops, to complex modifications incl. gene regulation using classical transgenesis and/or Genome Editing

Many scientists would not consider this kind of work as being synthetic biology

Only a few products (e.g. Flax for medical fiber) have been tested in Europe

Changes in a plant’s fitness by altering transcription factors require intensive exploration before any market relevance can be considered

Introducing improved carbon fixation systems (photosynthesis) in higher plants are currently basic research level.

SynBio in plants is still rare, therefore commercialization (time and products) is difficult to predict
<table>
<thead>
<tr>
<th>Technology(s)</th>
<th>Description of the new trait (phenotype characteristics)</th>
<th>Plant Species Name/Crop</th>
<th>Level of complexity of the SynBio development (low, medium, high)</th>
<th>Level of complexity of the SynBio product (low, medium, high)</th>
<th>Degree of molecular modification: number of endogenous genes modified; deletions, insertions, mutations (bp)</th>
<th>Final product</th>
<th>If relevant, comment on the comparator (currently used in conventional GMs)</th>
<th>Information on field trials, if relevant (e.g. small-scale/single year vs. large-scale/multiple years)</th>
<th>Geographic region (i.e. US, Canada, Australia, China, Japan)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transgenes</td>
<td>Plants/plant cell cultures producing food additives - e.g. colour.</td>
<td>any plant</td>
<td>medium</td>
<td>low. Downstream processing needed</td>
<td>1-5</td>
<td>n/a (in future iterations the plant chassis may also be edited e.g. to increase product yield)</td>
<td>Purified or semi-purified extract. There will be no transgenic plant released</td>
<td>wild-type cell cultures, plants</td>
<td>NO FIELD RELEASE - Cell culture, greenhouse</td>
</tr>
<tr>
<td>Transgenes</td>
<td>Plant food feedstocks engineered for increased nutrition - carotenoids e.g. astaxanthin</td>
<td>any crop</td>
<td>medium</td>
<td>low</td>
<td>1-10</td>
<td>n/a (in future iterations the plant chassis may also be edited e.g. to increase product yield)</td>
<td>Plant material/grains/seeds</td>
<td>wild-type plants</td>
<td>not yet (as far as we know)</td>
</tr>
<tr>
<td>Transgenes</td>
<td>Plant food or feedstocks engineered for increased nutrition - PUFAs (polyunsaturated fatty acids)</td>
<td>oil seed</td>
<td>medium</td>
<td>low</td>
<td>5-10</td>
<td>n/a (in future iterations the plant chassis may also be edited e.g. to increase product yield)</td>
<td>Plant material/grains/seeds</td>
<td>wild-type plants</td>
<td></td>
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<tr>
<td>Transgenes</td>
<td>Plant food or feedstocks engineered for increased nutrition - Amino acids, vitamin, proteins</td>
<td>any crop</td>
<td>medium</td>
<td>low</td>
<td>1-5</td>
<td>n/a (in future iterations the plant chassis may also be edited e.g. to increase product yield)</td>
<td>Plant material/grains/seeds</td>
<td>wild-type plants</td>
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<tr>
<td>Genome editing</td>
<td>Deletion of undesirable gene products, allergens - e.g. gluten</td>
<td>cereal</td>
<td>med</td>
<td>low</td>
<td>35-45 genes with point mutation in each (obtained with a single editing event)</td>
<td>Plant material/grains/seeds</td>
<td>iterations the plant chassis may also be edited e.g. to increase product yield</td>
<td></td>
<td>USA &amp; EU</td>
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</table>
Plant Expert’s Mapping of plant SynBio developments

- Technology:
  - Transgenes
  - Genome editing
- New trait:
  - Plant food feedstocks for increased nutrition
    - Carotenoids, PUFAs, low gluten
  - Plants with engineered complex traits
    - Yield, tolerance to biotic and abiotic stress
- Number of genes introduced:
  - 1-10, 5-10, Cas9 +sgRNAs introduced (not present in final product), 1-20
1. Plant food/feedstock for Increased nutrition; introducing Vitamin B12 pathway in plants (>15 genes)

https://doi.org/10.1038/nchembio.1086

2. Deletion of undesirable gene product e.g. Allergenics – low gluten by CRISPR mutagenesis


3. Plants with engineered complex traits – immunity. Addition of synthetic resistance genes (endogenous resistance proteins engineered to respond to an expanded range of pathogen proteins AND CRISPR mutations of endogenous susceptibility genes)

https://www.biorxiv.org/content/10.1101/611152v1
Scientific opinion on the adequacy of the guidance for ERA of GM plants, including MC;

Scientific opinion on the adequacy of the guidance for category 4 GMMs for MC and ERA;

By end of 2020, publication of final opinion after public consultation

In a later phase of this mandate other guidances will be assessed for their applicability for SynBio applications
Progress of SynBio Plant ERA WG

- **SynBio mapping exercise**
  - January 2019: Kick off WG activities
  - March 2019: JKI first results and Draft Scientific Opinion: how to report Guideline and Regulatory data requirements

- **JKI Procurement**
  - Selection of 3 cases

- **Scientific Opinion**
  - April-September 2019: Case study selection and Draft Scientific Opinion
  - October/November 2019: Finalize Draft Scientific Opinion for first reading at GMO Panel meeting

- **March 2020**
  - Public Consultation

- **Jan/February 2020**
  - GMO Panel endorsement

Next core meetings:
- June 20
- October 25
Acknowledgements

- **EFSA’s SC and SCER Unit: project coordination**
  - Caterina Barasso (GMM WG Contact point)
  - Reinhilde Schoonjans

- **EFSA staff: project contribution**
  - Jamie Aguilera (ERA, GMM)
  - Support Units: ALPHA, PPR, NUTRI, FEED, FIP and GMO

- **GMO Unit: Plant SynBio ERA coordination:**
  - Yann Devos (ERA)
  - Nikoletta Papadopoulou (MC, Plant ERA Contact point)
  - Tommaso Raffaello (MC, GMM)

- **GMO experts:** Ewen Mullins (chair), Josep Casacuberta, Tamas Dalmay, Antoine Messean, Adinda De Schrijver

- **Plant SynBio ERA WG Experts:** Nicola Patron, Matias Zurbriggen
### Composition of Working groups

- **GMM WG**

<table>
<thead>
<tr>
<th>Experts</th>
<th>Name</th>
<th>Role</th>
<th>Declaration of Interest</th>
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<tr>
<td></td>
<td>COCCONCELLI Pier Sandro</td>
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<td>Doi</td>
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<td></td>
<td>GLANDORF Poel</td>
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<td>HERMAN Lieve</td>
<td>Member</td>
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<td>TEbbe Christoph</td>
<td>Member</td>
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<td></td>
<td>DE LORENZO Victor</td>
<td>Hearing Expert</td>
<td>Doi</td>
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- **Plant SynBio ERA WG**

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<td>CASACUBERTA Josep</td>
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<td>DE SCHIUVER Adinda</td>
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<td>MESSEAN Antoine</td>
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<td>PATRONE Nicola</td>
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<td>ZURBRIGGEN Matias</td>
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