Results of the public consultation on the Scientific Committees' preliminary Opinion on Synthetic Biology II– Risk assessment methodologies and safety aspects

A public consultation on this Opinion was opened on the website of the non-food scientific committees between 19 December 2014 and 3 February 2015. Information about the public consultation was broadly communicated to national authorities, international organisations and other stakeholders.

20 organisations and individuals (providing in total 72 comments) participated in the public consultation providing input to different chapters and subchapters of the Opinion. Among the organisations participating in the consultation, there were universities, institutes of public health, industry representatives, NGOs and public authorities.

Each submission was carefully considered by the Scientific Committees and the scientific opinion has been revised to take account of relevant comments. The literature has been accordingly updated with relevant publications.

The Scientific Committees thank all contributors for their comments and for the references provided during the public consultation.

*The table below shows all comments received on different chapters of the Opinion and SCENIHR’s response to them. It is also indicated if the comment resulted in a change of the Opinion.*
**Comments received during the public consultation on the SCs preliminary Opinion II on "Synthetic Biology – Risk assessment methodologies and safety aspects"**

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of individual/organisation</th>
<th>Table of content to which comment refers</th>
<th>Comment</th>
<th>Scientific Committees Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fears Robin, European Academies Science Advisory Council (EASAC), <a href="mailto:RobinFears@aol.com">RobinFears@aol.com</a></td>
<td>3. SCIENTIFIC RATIONALE</td>
<td>EASAC welcomes the commitment shown by the European Commission in publicly consulting on synthetic biology and the thoughtful manner in which the issues have been presented relating to risk governance and its implications (sections 3.2 and 3.3). A number of points relevant to this second part of the consultation on synthetic biology have already been discussed in the EASAC response to the first part and, in further detail, in previous work published by EASAC (Report 2010 on <a href="http://www.easac.eu/fileadmin/PDF_s/reports_statements/Synthetic%20Biology%20report.pdf">http://www.easac.eu/fileadmin/PDF_s/reports_statements/Synthetic%20Biology%20report.pdf</a>). A recent Statement by IAP, the global network of academies of science, on synthetic biology (2014 on <a href="http://www.interacademies.net/File.aspx?id=23974">http://www.interacademies.net/File.aspx?id=23974</a>) addresses some relevant matters for this consultation, for example relating to: determining how to regulate; engaging with the public; preparing researchers; issues for education and scientific responsibility. We recommend that you consult these sources and while, at this stage, we will not repeat the relevant analysis and conclusions from the previous EASAC and IAP work, we emphasise the critical importance of developing coherent, science-based, regulations. We now confine our response to noting the importance of some strategic issues: 1. Potential value of synthetic biology research. In addition to the sector-specific applications listed in the consultation document (section 3.1.1 and Table 2), we want to emphasise the value of synthetic biology research as a tool to aid the understanding of natural processes. One example of how synthetic biology is</td>
<td>1. No change in the Opinion is required. The SCs read the paper by Pál, Papp and Pósfai, and think it is not relevant for our purpose. They discuss the possibility of expanding directed</td>
</tr>
</tbody>
</table>
important in the experimental repertoire to understand how living systems work is provided by the recent publication of Pal, Papp and Posfai, “The dawn of evolutionary genome engineering” (Nature Reviews Genetics 2014 15, 504, on http://www.nature.com/nrg/journal/v15/n7/index.html#close).

2. Public engagement. EASAC welcomes the commitment to openness and public engagement (section 3.2.1). In our work we published a separate lay summary (http://www.easac.eu/home/reports-and-statements/detail-view/article/synthetic-bi-1.html), that has now been translated into many European languages, and that you might find useful as a resource.

3. Governance and innovation. In section 3.2, when discussing the literature on responsible governance and innovation, we suggest that you may wish to refer to the report of the UK Government Chief Scientist, “Innovation: managing risk, not avoiding it” (2014 on https://www.gov.uk/government/publications/innovation-managing-risk-not-avoiding-it), that includes synthetic biology as a case study.

4. “DIY synthetic biology”. The emergence of citizen science (consultation page 36) was also discussed in the EASAC 2010 report and, as the consultation recognises, raises issues for scientific responsibility and personal/institutional codes of conduct. This aspect is discussed further in the IAP Statement and previous IAP work on responsible science.

5. Engineering biosafety. It would be useful for the discussion of “biocontainment” for building in additional safety features (sections 3.5 and 3.6) to take account of the most recent evidence. For example, the work published in the journal Nature (Dolgin, Nature 21 January 2015 “GM microbes that can't escape the lab”), that engineers additional safety by creating dependency on an amino acid that does not occur in nature.

6. Biosecurity. We note that issues for “biosecurity” (relating to intentional misuse) are judged to be mainly outside the scope of this Opinion but it is important for the EU to consider how risk assessment procedures might need to cover biosecurity as well as biosafety issues. We suggest that the Opinion provides a link on how and where the matters are considered.

evolution approaches to the genome scale, but there is little to learn here for our assessment of risks.

No change in the Opinion is required.

3. This reference has been added to the Opinion.

5. The work by Mandell et al. and Rovner et al. on this new development has been added.

6. Although biosecurity is an important consideration in this context, it is outside the focus of this Opinion.
The European Federation of Biotechnology (EFB) is Europe’s non-profit federation of National Biotechnology Associations, Learned Societies, Universities, Scientific Institutes, Biotech Companies and individual biotechnologists working to promote biotechnology throughout Europe and beyond. The mission of EFB is to promote the safe, sustainable and beneficial use of the life sciences, to promote research and innovation at the cutting edge of biotechnology, to provide a forum for interdisciplinary and international cooperation, to improve scientific education and to facilitate an informed dialogue between scientists and the public. With more than 100 Institutional members from across Europe and more than 30,000 personal members, the EFB has 14 Regional Branch Offices in Europe to support its activities in the various areas of biotechnology covered by the Federation. The EFB welcomes the opportunity to comment on the preliminary opinion developed by the Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and Scientific Committee on Consumer Safety (SCCS). Nevertheless, given the importance of this issue, which is at the heart of Life Science research and development, it is unfortunate that this consultation coincided with a major international holiday period and thereby limited the possibility for preparing comments. Not having commented on the first opinion, some of the EFB’s comments may relate to more fundamental aspects of definition, scope and the justification for requiring risk assessment, which the EFB feels were misrepresented in the first opinion.

The EFB and its member associations have been, and will continue to be, involved in very diverse advances and applications of ‘Synthetic Biology’, for which the scientific progress and diversification hardly allows a simplistic definition. In fact, it is questioned as to whether such a definition is required. If the only purpose were to be to delineate a group of materials that thereby are subjected to regulatory oversight, the effort would potentially miss its main goal, i.e. to identify potential risks that require management. It would not be in line with the precautionary principle, which it advocates to implement. The precautionary principle as presented in the Commission’s Communication (2000), requires that measures should clearly be proportional to the threat and fairly handle all elements...
that present similar risks. This SC preliminary opinion largely investigates hypothetical or unknown risks related to a broad range of diverse technologies, being lumped together as SynBio. While the EFB subscribes to the precautionary principle as an excellent guidance for responsible research, it is unclear why all comparable biologicals should not be subjected to the same level of scrutiny.

EFB calls for careful and scientifically justified communication in order to inform the public adequately and avoiding the that Synthetic Biology is creating dangerous organisms. The cell factory approach is already being used and is successfully producing molecules of commercial interest using hosts such as compromised strains of E.coli, yeast etc. that are safe and already being used to overexpress proteins and in many applications. Synthetic biology is using known molecular biology techniques and developing these for the process in hand. Synthetic biology can be very broad and each case needs to be looked at for its risk. Most of the genetically modified products would come under standard GMO regulations and are of relatively low risk.

The same as a comprehensive risk assessment, does not imply that all developments in SynBio pose the same level of risk, but rather tries to identify areas that might require closer scrutiny.

The SCs agree that “Synthetic biology can be very broad and each case needs to be looked at for its risk” and that “Most of the genetically modified products would come under standard GMO regulations” as it is explicitly stated in the Opinion.

| 3. Carron Delphine, 'EuropaBio, d.carron@europabio.org,  |
| 3. SCIENTIFIC RATIONALE |
| GENERAL |

We highlight several areas of agreement:
- We agree with the overarching comments that the framework for risk assessment of new applications resulting from synthetic biology may be addressed using current methodology used for chemical and biological fields. Because these methodologies are based on the risk assessment paradigm put forward by NAS (Risk Assessment in the Federal Government: Managing the Process, i.e., the "Red Book"), are broadly accepted and are applicable to products from many technologies, there is no need to develop a new framework.

- We further agree with statements in the Opinion suggesting that new or novel products resulting from practicing synthetic biology may in certain instances require new input data be developed for a risk assessment. These data would reduce uncertainty associated with applications that have not been time-tested.

Our major remarks/points of disagreement:
- Before the applicability of existing risk assessment methodologies to Synthetic Biology can be evaluated in a meaningful way, there is a need for a precise self-contained, science-based definition of synthetic biology which appropriately considers the scope and

The SCs remarked on a contradiction in this comment, on one hand it is argued that "We agree with the overarching comments that the framework for risk assessment of new applications resulting from synthetic biology may be addressed using current methodology used for chemical and biological fields." and on the other hand it says: "We encourage a product-based, not a process-based risk assessment approach" that would require a total change in the way the assessment is done. No change in the Opinion is required.

Part 1 of the Opinion clarifies that SynBio is a result of accelerated and facilitated ways towards GMOs, which potentially result in new risks. It is the aim of this part of the Opinion to assess to which extent this is indeed the case and which of the very diverse areas covered by SynBio are in need of closer scrutiny.
degree of novelty of such products. The synthetic biology definition should focus on specificities that go beyond those of products already used and potentially regulated today. This would include clarifying the extent of genetic modification (e.g. SNP, single gene, chromosome, whole genome) and novelty of the genetic construct or gene product needed for this modification to be considered as following a synthetic biology approach and not genetic engineering.

- It would not be appropriate to include GMOs and New Breeding Technologies (NBTs) under the umbrella of synthetic biology. Risk assessment methodologies for GMO risk assessment are well established and do not require re-evaluation in the ambit of Synthetic Biology.

- We encourage a product-based, not a process-based risk assessment approach. Any potential risks would derive from the composition of a product, not from the process used to make it.

- The term “manipulation” is used throughout the document of the EC. In our view, this term has a negative connotation (in combination with synthetic biology). So we suggest to replace the term “manipulation” by “modification”.

- Regarding the use of the word “SynBio” to represent Synthetic Biology, we suggest to not use an abbreviation but use the full term Synthetic Biology consistently throughout all documents. The term Synthetic Biology is short enough.

- We disagree that gene modification can be accelerated, as is noted many times throughout the Opinion. Tools that advance the efficiency of biological technologies are not focused on altering genes more quickly, but rather on increasing the scale and precision of genetic modifications.

- Further to that point, we do not agree that the increased advancement of modifications by new technologies will result in unforeseen or additional risks that would require new risk assessment methods. The efficiency of a process does not introduce additional risk into the resulting product.

- We do not believe a "no risk” policy should apply to synthetic biology, as implied by the questions imparting a requirement of inherent safety to its use. With no other technology do we apply a

No change in the Opinion is required. See the answer above.

The SCs agree and replaced “manipulation” by “modification”.

The SCs think that the consistent use of the abbreviation SynBio contributes to the readability of this document.

The SCs consider that genetic modifications now can often be introduced much faster than before (the increase in scale is a direct result of this acceleration).

The aim of this Opinion is to assess whether or not this is correct.

The questions were decided in the mandate, which itself is based on broader discussions of
zero risk standard. Advanced biotechnologies can be used in a safe manner and can also be used to provide innovative solutions to societal needs. To avoid the potential for lost opportunity resulting from unnecessary constraints, any safety locks should be proportional to the potential risk associated with a given application. The field, e.g. as part of the Convention on Biological Diversity.

| 4. | Carron Delphine, 'EuropaBio, d.carron@europabio.org, | 3. SCIENTIFIC RATIONALE | - We do not think it is appropriate to include “Citizen Science” in the synthetic biology discussion and the section should be removed from the document. It cannot be described ... as having “evolved from genetic engineering” (page 11, line 28-29), nor are the “established mechanisms governing genetic engineering” (page 13, lines 15-16) relevant. The capacity and capabilities of amateur scientists/societies is highly speculative, and the regulation of such activities and how they are enabled are separate issues to risk assessment for synthetic biology.

Specific Comments:
- Page 5 Line 24-27: “six novel SynBio developments...” Based on which criteria were these 6 categories developed? In Opinion I, while elaborating on the operational definition of SB, these specific 6 categories are not listed. In addition, there is inconsistency in the choice of developments, with some of the listed points clearly not being "novel". For example, DNA synthesis and genome editing are not novel approaches (item 5) nor are DNA part libraries and methods (item1). If novelty is to be addressed, more precision in description of what is meant under the 6 chosen items will be welcomed. Our concern is that the list is very biased, and does not seem to achieve sufficient focus related to novelty and what goes beyond the current state of the art in genetic engineering technologies. As a conclusion, we do not support the choice the SCs made in listing these 6 categories, which in effect is a de facto inclusion list, that again will generate a lot of focus on the technology rather than the product.

- Page 5 Line 29-30: “lack of ‘comparators” is put forward as a major challenge in the risk assessment of potential products of synthetic biology – however it is not the case that comparative assessment is needed in all cases and this needs to be made clearer in the text. Further, current Implementing Regulation 503/2013 as well as...
Codex Alimentarius foresee a situation where there is a need to complement the comparative assessment in case where the non-GM parental line might be considered not a sole appropriate comparator. Hence this aspect of risk assessment of Synthetic Biology products is already covered in the scope of current GMO legislation and the argument of “lack of comparators” is considered not to be a valid one.

- Page 6 Line 18 – 19: Regarding “acceleration of GM processes will require new methodological approaches”, it is unclear why the SC believe that the acceleration of the genetic modification process will be a challenge to risk assessment and why this point is brought forward as a challenge.

- Page 6 Line 23 – 24: “Though present risk assessment methodologies are appropriate for assessing potential risks of SynBio activities and products,...” is welcomed as it recognises that current RA are applicable to future products of synthetic biology.

- Page 9 Line 19 – 21: The precautionary principle is well defined in EU legislation and makes no reference to “components and products generated by SynBio”. Suggest aligning language more appropriately with EU legislation.

- Page 9 Line 28: Suggest providing a definition of “synthetic genomics” and how it fits with the mandate.

- Page 9 Line 31 – 32: Please provide scientific justification why and in which way “synthetic genomics/SynBio differs from previous gene modification techniques”

Opinion I as:

“The step-by-step process is a concept that guides the introduction of GMOs into the environment. It involves the gradual reduction of the containment of GMOs and an increase of the scale of release, step-by-step, but only if evaluation of the earlier steps in terms of protection of human health and the environment indicates that the next step can be taken (Directive 2001/18/EC).”

Advances in GM through SynBio might lead to a situation where the parental line does not even exist. This scenario needs to be addressed.

P6/18: This comment refers to the abstract of the Opinion. The main text explains this in detail.

The SCs mean that the regulatory bodies will be overwhelmed by the number of modifications if biosafety boards/regulators continue to use the old model.

P6/23: True, but the SCs also investigated when this was not the case.

P9/19: The scope here is wider than the EU legislation, thus the SCs decided to keep the language as is.

P9/28: Synthetic genomics and its relationship to SynBio are explained in Opinion 1 (see Opinion 1, section 3.3.1.3).

P9/31: This evidence is provided in Opinion 1.
| 5. | Carron Delphine, 'EuropaBio, d.carron@europabio.org, | 3. SCIENTIFIC RATIONALE | We have submitted our comments in the different sections. For your convenience, a complete overview of all our comments can be found in attachment. Many thanks. | No change in the Opinion is required. |
| 6. | de Lange Orlando, 'University of Tübingen, immununichwork@gmail.com, Germany | 3.1 Introduction | It's not clear whether Table 1 includes all classical-GM products. I guess it does based on Opinion I which subsumes GM within SynBio. I think that runs counter to expectations, at least for a reader within the scientific community and should be made explicit. | Table 1 is specific for SynBio as understood by the authors of the cited publication; it is not based on the definition in Opinion I. |

Pg.11 ln 26. I find it misleading to suggest that SynBio has grown to include many fields. It is rather the case that many fields that all arose in parallel around the same time (protocells, synthetic biological circuitry and massive-scale DNA synthesis) and ended up grouped together under the term Synbio. (O'Mailey, 2008). This is a comment more suited to Opinion I and therefore too late but I also think it's important to bear in mind when talking about Risks. SynBio is a set of practices that are not inherently connected and nor are their risks or necessary risk limitation and management procedures. Having said that I think this situation is reflected in the approach taken in this opinion, treating each sub-field of SynBio separately. |

Pg. 13 ln. 3: The report Marshall et al. 2014 refers to crops created using classical GM methods, not any of the methods discussed in this report and therefore I consider it disingenuous to include it in the list of 'SynBio accomplishments'. Might I suggest Lin et al. (Nature, 2014, A faster Rubisco with potential to increase photosynthesis in crops) which used synthesised gene cassettes, a modular approach and a fairly large scale genome reengineering. Or Antunes et al, creating an orthogonal biosensor inside Arabidopsis plants. |

Pg. 13 ln. 10: The SCs agree. This is part of the area identified for further research in the forthcoming Opinion III. |

P11/l26: A broad set of technologies, methods and concepts that expand the scope and scale of genetic modifications are part of the core concept of SynBio. |

The SCs agree. According to our definition, the boundaries are blurred, the plants discussed in this article were developed years ago, even though they are only entering the market now. The reference was deleted. The SCs included Lin et al. and Antunes et al. |

Pg. 13 ln. 10: It is commonly stated that altering cellular components can have unexpected effects but I think it's really important to try and clarify and understand this in order to be able to make better risk assessments. At the very list address what the 'Known unknowns' are i.e. what processes are commonly perturbed and what processes are very stable and can be ignored for risk assessment. This, I think could be a worthwhile question to focus some research on. |
Table 2. I have uploaded our products inventory list. This list will be updated and converted into an interactive online database, which will be available in April 2015.

The SCs are aware of this list, but only a draft version is published to date. The reference list was updated accordingly. http://www.synbioproject.org/inventories/applications_inventor/

The definition of SynBio in Opinion I emphasises that there indeed is a "continuum of technologies that are using biological systems". But the implications of SynBio cannot be assessed fairly by ignoring the heterogeneity of the field. In contrast to the view of the EFB, the Opinion makes a clear effort to assess developments separately and with the justified care, rather than "lumping everything in one approach".

In the Opinion each development is indeed "evaluated on its own merits and possible applications, its potential risks and benefits". It is not the choice of the SCs to lump diverse fields of science together, but it would be ludicrous to ignore the major scientific consensus that SynBio includes a broad set of technologies. See Opinion I for a comprehensive evaluation of previous definitions of SynBio. In any case, the SC does evaluate the various developments one by one.
While examples of novel developments can be useful, providing broad categories is an oversimplification and can lead to scientifically unjustified decisions. In fact indicating that these are SynBio developments and novel, is misleading and incorrect.\footnote{p. 11/ l. 30}

It is not clear why Citizen science (e.g. Do-It-Yourself Biology DIYBio) is included in this list of technologies. EFB understands that citizen science is defined by who conducts scientific work in what environment (and under what controls). It is therefore not a technique per se. In fact citizens can potentially perform any project provided they have access to technical means. There are obviously pertinent questions on education, safety, security, compliance and oversight that need to be addressed, but the concerns are likely not different than for academic or industrial uses. Including it in this list is confusing and seems to be intended to create a sense of loss of control.\footnote{p. 11/ l. 35}

A statement on the limited ability to engineer predictable outcomes of biological systems can only be justified by the broad scope of SynBio. For specific techniques this may be very different and highly predictable. It is incorrect and discriminatory to use such generalised statements, only because for certain cutting-edge applications the predictability may be less established.\footnote{p. 13/ l. 07 (and following sentences)}

While the SCs agree that SynBio is based on a continuous development from previous GM technologies, Opinion I provides detailed evidence for considering developments in each of these areas as novel and relevant.

The SCs agree and the text was revised to reflect that citizen science is a development and not a technology.

The Opinion states that “the ability to engineer predictable outcomes of biological systems remains embryonic relative to most other fields of engineering” – this is a general consensus amongst experimental biologists, and there are no exceptions to this statement, which is independent of the specific techniques used. Synthetic Biology remains far from being a true engineering discipline.
| 9. | Van der Vlugt Cecile, 'National Institute of Public Health and the Environment, Cecile.van.der.Vlugt @rivm.nl, | 3.1 Introduction | p. 11, l. 30 The working group proposes a subdivision of SynBio technologies for which the risk assessment methodology is discussed. A number of developments within the realm of synthetic biology seems not to be covered by this subdivision. For example: abiotic synthetic biology, developments in computer aided design (CAD), random design of genes/genomes, and DNA-origami seem not to be covered. We ask the working group to explain why these technologies are not included. |
| | | | p. 11, l. 35 Citizen science is put forward by the working group as a development which should be addressed. We agree that this development should be followed closely. However, risk assessment for DIY does not seem to be fundamentally different from 'regular' risk assessment. Additionally, more general developments of which citizen science is one of the consequences could be identified. In our opinion two fundamental developments underlying this are: 1) proliferation of the concept, technique, toolbox and use of synthetic biology and 2) the aspect of the level of knowledge on risk and security issues with the expected users. We ask the working group to reflect on a) whether or not 'citizen science' should be discussed as a separate item and b) whether or not the discussion focus of the working group should instead be on other more fundamental underlying developments. |
| | | | These developments are not included because they are not SynBio according to our very broad and inclusive definition. Specific reasons are provided in Opinion I (e.g. section 3.2. of Opinion and exclusion criteria p27 of Opinion I). Several of the areas mentioned do not even exist, e.g. “abiotic biology” and “random design” and perhaps the intention was to include these areas: prebiotic world, and directed evolution. The SCs have reflected on the proposed questions and the answers are (a) "yes, it should" and (b) “no, it should not”. The discussion of DIYbio in the Opinion is sufficiently broad to cover the fundamental underlying developments, rather than being restricted to isolated sociological phenomena. |
Carron Delphine, 'EuropaBio, d.carron@europabio.org,

3.1 Introduction

- Page 11 Line 12-14 + table: Regarding the sentence: “An update by BCC Research in 2014 stated that the overall market is expected to grow ... from 2013 to 2018.”, taken together with Line 19 -21: “The dawn of SynBio was in January 2000, when two articles were published ...” It seems contradictory to first provide data on the synthetic biology market which apparently is already well established and then follow with a statement that SB starts in 2000 as a research.

Recommendation: consider clarifying why products are categorised as synthetic biology, and whether these are meeting the definition and understanding put forward by the SCs.

- Page 11 Line 26 – 35: the statement “Many of these technologies and methods evolved from genetic engineering and include...” is factually incorrect as it is not possible to draw a line between genetic engineering and synthetic biology, especially when the authors add in the list of “evolved technologies” DNA synthesis and genome editing – which is an established technology with long history of use, as well as adding DIYBio, which by itself can be anything that is practiced outside of established laboratory environment, and is not specifically and exclusively novel and synthetic biology.

Page 12, lines 3-14 and page 13, lines 1-5: The achievements mentioned are intended to illustrate the self-contained character of Synthetic Biology. However, there is a strong overlap between synthetic biology and classical genetic modification technologies putting the presented examples in question.

- Page 13 line3: Please provide a scientific justification/rational why the production of drought tolerant crops is presented as an achievement of Synthetic Biology. We disagree, since the methods (plant transformation) and tools (genetic elements with history of safe use) used to obtain drought tolerant crops are standard practice in the GMO area.

- Page 13, lines 10-11: Synthetic Biology is not a unique field of engineering because it is not clearly defined and strongly overlaps with classical gene modification technologies.

The SCs do not consider this a contradiction: this research is just particularly fast in being translated to marketable products. The difficulties of discriminating between SynBio and classical GM were discussed extensively in Opinion I. The BCC Research data are not based on the SCs definition, but the categorisation seems to be similar. There are 13 years between 2000 and 2013.

Why would the fact that it is impossible to draw a line make it factually incorrect to state that there has been evolution? With regard to DIYBio the SCs now marked it as a significant development (and not a technology).

The SCs are aware of this overlap, and this is and was extensively discussed, both in the present Opinion and in Opinion I. However, this does not put “the presented examples in question”.

The SCs agree. Please read also the answer to comment 6.

Plant drought tolerance requires several genes and pathways, so again the question is: where does GM end and SynBio start? The SCs deleted this example.

Classical gene modification technologies would not be considered a field of engineering by most practitioners.
| 11. | Elbing Kerstin, 'German Life Science Association (VBIO e. V.), elbing@vbio.de, | 3.1 Introduction | Page 11, line 30ff
The SCs preliminary Opinion discusses risk assessment classified as „technologies and methods (mostly) evolved from genetic engineering“. Given this background structure, the inclusion of Citizen Science (e.g. Do-It-Yourself Biology - DIY Biology) seems misleading as it is neither a technology nor a method of engineering. Citizen Science is specified by the Person doing Science (Synthetic Biology) in a certain framework of facilities apart of professional research infrastructures. We agree that DIY Biology raises questions on training, safety, security, compliance and supervision that need to be addressed. But as a matter of rigidity we recommend to include all special consideration concerning Citizen Science under 3.2 Risk governance (page 14ff).

The SCs agree with this comment and the sentence has been edited accordingly. The SCs prefer to keep the Opinion on citizen science in one section, but included a reference to this subject in section 3.2 on risk governance. |
| 12. | de Lange Orlando | 3.2 Risk governance |
| | | |
| pg 15 ln 40: I know that this opinion deliberately does not address ethics but I found point 1. of your list of ethical quandaries very odd. To my mind Synthetic Biology CANNOT blur the boundary if the boundary is real and objective. If the boundary is simply a thought construct then is it not good to refine the definition? SynBio will help us clarify where we want to place the boundary. In addition point 2 on the list is confusing, does it refer only to intentional release or does it imply that SynBio in a lab interferes inherently with nature?
Points 3. and 4. on the list seemed very reasonable to me. |
<p>| The SCs agree and the text of the Opinion was amended accordingly. Old text: 'There are four main generic ethical considerations. SynBio developments may:' New text: &quot;There are four main generic ethical considerations which are often raised in the context of debates on SynBio. SynBio developments might:&quot; |</p>
<table>
<thead>
<tr>
<th></th>
<th>Name and Affiliation</th>
<th>3.2 Risk governance</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.</td>
<td>Oleksiewicz Martin, 'Centre for Biosecurity and Biopreparedness, <a href="mailto:mbol@ssi.dk">mbol@ssi.dk</a>,</td>
<td>We at CBB welcome the initiative from the European Commission to ensure regulatory biosafety structures for the emerging field of synthetic biology. We believe the Opinion comprehensively describes challenges that synthetic biology development may pose to biosafety practice in the EU, and proposes very sound actions to address potential challenges through the next 10 years. We note that the Opinion states that &quot;Although biosecurity cannot be ignored here, it is not the main focus of this Opinion&quot;. However, we have been unable in the Opinion to find any discussion of biosecurity implications from potential misuse of synthetic biology. We suggest that there is a real need to develop strategies to address biosecurity issues in the highly complex field of synthetic biology, and hope this issue may yet be covered in the planned series of Opinions on synthetic biology.</td>
<td>Scientific Committees agree with the importance of 'biosecurity' but this was outside the scope of the current mandate. A new sentence was included in the Opinion to clarify this point: &quot;Although biosecurity is an important consideration in this context, it is outside the focus of this Opinion.&quot;</td>
</tr>
<tr>
<td>14.</td>
<td>European Federation of Biotechnology (EFB), <a href="mailto:efb@efb-central.org">efb@efb-central.org</a>,</td>
<td>It is not at all clear why a brief discussion on social, governance and ethical implications of SynBio is needed to fully appreciate the understanding of risks of SynBio. This statement suggests there are risks that can only be seen in the light of the 3 areas. The further superfluous elaboration of these topics is not supporting such suggestion. p. 15/l. 35 Again this is a false argument as the problem is entirely due to the broad SynBio definition proposed by the SC. Most specific applications will be straightforward to evaluate, as indeed confirmed on p. 16/ l. 2 &quot;.. and none of these individual concerns is unique to SynBio. Thus, the question is whether the summation of these considerations for SynBio constitutes a 'unique' ethical concern.” The Opinion covers the broadest possible range of SynBio applications, and the SCs cannot accept the suggestion that it be restricted to straightforward “specific applications”. It would be inappropriate to restrict the scope of the analysis to such a degree that it does not cover even the most generally accepted components of the SynBio developments (even in its present form, some areas that might arguably belong to SynBio under the even broader definitions of other scientists are excluded).</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Van der Vlugt Cecile, 'National Institute of Public Health and the Environment, Cecile.van.der.Vlugt @rivm.nl,</td>
<td>For what reason the Working Group provides considerations about ethics and governance of SynBio in this opinion, as it is outside the scope of the mandate? It is unclear what the contribution of this paragraph 3.2 is to risk assessment methodologies in the context of the Directives 2001/18/EC and 2009/41/EC. When keeping the text it should be clearly clarified why this text is important for this scientific SCENHIR opinion on risk assessment. As explained in the Opinion, this background information &quot;is needed to fully appreciate the understanding of the risks of SynBio”. Risks cannot be assessed without reference to the broader context of risk governance.</td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th></th>
<th>Carron Delphine, 'EuropaBio, <a href="mailto:d.carron@europabio.org">d.carron@europabio.org</a>,</th>
<th>3.2 Risk governance</th>
<th>- Page 15 line 4 – 6: There is a contradiction between both sentences, “those involved in SynBio must be proactive”, but at the same time this “process needs to be independent of synthetic biologists”.</th>
<th>The SCs agree with the comment. These two sentences have been removed from the final version of the Opinion.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paton Michael, 'Health &amp; Safety Executive, <a href="mailto:michael.paton@hse.gsi.gov.uk">michael.paton@hse.gsi.gov.uk</a>,</td>
<td>3.2 Risk governance</td>
<td>Have the SCs considered the points raised in the &quot;Innovation: Managing risks, not avoiding it&quot;, which is the first annual report of the UK Government Chief Scientific Adviser (Sir Mark Walport), in which he looks at approaches to risk in the context of innovation. The supporting evidence contains the views of leading experts looking at risk and uncertainty from a wide range of perspectives, including: social; psychological; industrial; and financial. A series of case studies illustrate its core themes including one on synthetic biology. The essential point is that for innovation and emerging technologies there is a need to manage risk rather than avoiding it.</td>
<td>The SCs agree that this seems to be a rather obvious conclusion, and this essential point has of course informed the analysis underlying the Opinion.</td>
</tr>
<tr>
<td></td>
<td>Elbing Kerstin, 'German Life Science Association (VBIO e. V.), <a href="mailto:elbing@vbio.de">elbing@vbio.de</a>,</td>
<td>3.2 Risk governance</td>
<td>Page 14, line 29ff The preliminary opinion states that &quot;Risk governance can be at the level of authorities, but also ‘self-governance’ should be recognised as an important contribution toward safety&quot;. We agree with the opinion. However, we wish to indicate that both approaches might be in fragile relationship to each other. In fact, bureaucratic procedures issued by authorities may not enforce self-governance structures. No change in the Opinion is required.</td>
<td>The SCs agree with the importance of 'biosecurity' but this was outside the scope of the current mandate. Although biosecurity is an important consideration in this context, it is outside the focus of this Opinion.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Page 14, line 43/44 As biosecurity issues are not the main scope of the SCs, we understand that the SCs in its preliminary opinion focused on biosafety. Still, we would like to encourage the SCs and its members to actively participate in future discussions on biosecurity aspects potentially associated with Synthetic Biology. The SCs profound knowledge of Synthetic Biology will be needed there, too. In case of formal obstacles that prevent SCs to take part in the biosecurity discourse in EU bodies, we ask you to ensure that evidence-based scientific knowledge on Synthetic Biology will be carefully considered. The SCs agree and removed this sentence.</td>
<td></td>
</tr>
<tr>
<td>Page</td>
<td>Line</td>
<td>Text</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td>------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>16ff</td>
<td>Amongst others the preliminary opinion recommends to give consideration „to relevant education in schools“. We certainly understand that school curricula are national or federal matters. In terms of promoting best practice we would nevertheless recommend that the Commission supports suitable educational programs. This is beyond the scope of the SCs, but we would appreciate if the SCs would consider and advocate such a commitment in future discussions within the EU Commission.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>20ff</td>
<td>Synthetic Biology is quickly evolving and we support the opinion that this has to be reflected by academic education as well as by professional training. Courses should be accessible and affordable to a growing audience (also including DIY biologists). Training can be integrated into on-campus programs or offered as MOOCs, which will be suitable tools to ensure sufficient spread of knowledge. Within the framework of self-governance MOOCs might serve as an important tool. Considering the rapid developments within Synthetic Biology (and the corresponding MOOCs) it will hardly be possible for individual MOOCs to undergo a complex accreditation process. We alternatively suggest that the SCs encourages the initiation of a project to develop a set of minimal criteria for communication via MOOCs, or for training in the field of Synthetic Biology.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1-4</td>
<td>We support the opinion that the mentioned issues are „frequently raised following a major technological development and none of these individual concerns is unique to SynBio“. For us, the summation of these considerations does not justify a ‘unique’ ethical concern for issues related to Synthetic Biology.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**19. No agreement to disclose personal data**

3.3 Implications of SynBio for risk assessment

27 - 3.3.3 Risk Assessment Method. The below represents the opinion of the author and is not an official position of the European Chemicals Agency.

The SCs considered the comment unclear and decided that no change in the Opinion is necessary.
<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>20. Kuiken Todd, 'Woodrow Wilson Center', <a href="mailto:todd.kuiken@wilsoncenter.org">todd.kuiken@wilsoncenter.org</a>, USA</td>
<td>3.3 Implications of Synthetic Biology for risk assessment</td>
<td>I have attached a research article that addresses risk assessment of synthetic biology for consideration. I would also like to submit the following: <a href="http://www.synbioproject.org/site/assets/files/1374/synbio_res_agenda1.pdf">http://www.synbioproject.org/site/assets/files/1374/synbio_res_agenda1.pdf</a></td>
<td>Indeed a number of points raised in that paper were also discussed in the SCs, such as comparators, speed of change, information exchange systems, horizontal gene transfer. While physical and evolutionary principles are the same, the regulatory frameworks in the US and Europe are different, and that difference needs to be acknowledged when planning for international measures.</td>
<td></td>
</tr>
<tr>
<td>21. European Federation of Biotechnology (EFB), <a href="mailto:efb@efb-central.org">efb@efb-central.org</a>,</td>
<td>3.3 Implications of Synthetic Biology for risk assessment</td>
<td>The opening statement “In the safety assessment of SynBio, there is high complexity and uncertainty.” is another example of the effect of lumping diverse techniques that result in very different applications. The SCs have missed an opportunity to create clarity, rather than evading the mandate. p. 16/1. 6 It is not clear what is meant by focusing on “beyond the state-of-the-art SynBio technologies”. What does this mean for state-of-the-art SynBio technologies? Why does the remainder of the document include many references to state-of-the-art technologies? p. 16/1. 26; this sentence is indeed unclear and was edited as follows: “This identification focuses on those developments of SynBio technologies that move beyond the state of the art of genetic modification as practiced about 10 years ago”. p. 17/1. 28 The EFB stresses that this definition of Synthetic Biology is scientifically unfounded. We deplore that an important opportunity has been lost with which to focus on the product rather than on the method by which it is created. Furthermore, the definition creates legal vagueness and can only trigger further disputes. Finally, there is no indication that this definition identifies a group of products that poses significant new risks that are not already addressed in other legislation. p. 17/1. 28. It would be inappropriate to define SynBio as a product – it clearly is a collection of methods and concepts. The scientific basis for the definition is described in Opinion 1. It is unclear why the EFB would want a definition exclusively to “identify a group of products that poses significant new risks” – obviously not all products of SynBio will pose new risks.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p. 19/1. 09 It is stated that some pathogens and most GMOs are not classified into risk groups. It is common practice for biosafety practitioners to take the classifications as a starting point and to document the safety features determining the risk group. In this effort different internationally available listings can be used. Given the large diversity of potential GMOs, classifications are based on criteria. The fact that no risk group has been assigned should not be seen as a drawback, rather it highlights the need to have clear and scientifically justified criteria. Guidance for criteria can be found in p. 19/1.9: the text of the Opinion does not indicate that the lack of assignment to risk groups is a drawback; it just states an indisputable fact, and then goes on to list the criteria used for assignment to a risk group.
<table>
<thead>
<tr>
<th>Page</th>
<th>Name</th>
<th>Section</th>
<th>Line</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>Van der Vlugt Cecile, 'National Institute of Public Health and the Environment, <a href="mailto:Cecile.van.der.Vlugt@rivm.nl">Cecile.van.der.Vlugt@rivm.nl</a></td>
<td>3.3 Implications of SynBio for risk assessment</td>
<td>16, line 20</td>
<td>The literature reference of Baldo et al., 2013 is missing in paragraph 7.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17, line 1-2</td>
<td>The text mentions ‘potential hazards’ and adverse effects’. However, in the GMO risk assessment terminology ‘hazard’ is synonym for a ‘potential adverse effect’. The wording ‘potential hazard’ is therefore not appropriate. Please correct this in the opinion in a consistent way.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18 Figure 1</td>
<td>We do not agree with the title ‘Risk assessment of contained use’ for Figure 1. In this Figure a risk assessment methodology for biological risks is shown, e.g. for pathogenic microorganisms. A risk assessment for GMOs under contained use needs additional elements as shown in the text on p.19 line 29-33. The original title ‘Biological risk assessment’ as mentioned in the literature reference is preferred for this Figure, or this Figure should be left out.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18, line 16</td>
<td>This opinion is focused on the risk assessment methodologies as stated in the Directives 2001/18/EC and 2009/41/EC. It is unclear why Directive 2000/54/EC is mentioned in this paragraph. The risk assessment methodology for contained use in The Netherlands, for example, is exclusively based on 2009/41/EC in order to protect public health and the environment.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18, line 20</td>
<td>Risk assessment of contained use activities’ is mentioned here. Please note that ‘Biological risk assessment’ is meant here and not a risk assessment of a GMO since the risk elements mentioned on p.19, line 27-33, are not included!</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16, line 20</td>
<td>The reference was included in the reference list.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17, line 1-2</td>
<td>The SCs agree with the comment and the text of the Opinion was changed as follows: Old text: 'Potential hazard assessment depends on what is to be protected, where to protect it and over what time period' New text: 'the identification of hazard or potential adverse effect depends on what is to be protected, where to protect it and over what time period.'</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18 figure 1</td>
<td>The SCs agree with the comment and the title of the figure was changed to: 'Biological risk assessment for contained use activities’</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16 and line 20</td>
<td>We would like to refer to Opinion 1, p16, 4th paragraph under section 3.3.2.1. ‘While Directive 2009/41/EC only covers the contained use of GMMs, specific European Member States, such as Belgium, implemented the Directive into their national legislation by broadening the scope to include GMOs and pathogenic organisms for humans, animals and plants. In Switzerland, the Directive on contained use of GMMs served as the basis for the set-up of national legislation covering work...’</td>
</tr>
</tbody>
</table>
There are no risk classes of GMOs. There are risk classes of pathogens, and subsequently for 'recipient organisms' used for genetic modification.

In this opinion it would be sufficient to refer to lists of pathogenic microorganisms in order to determine their class of risk. For pathogens not listed, member states use different criteria (for example, all plant pathogens are listed as risk class 2 in The Netherlands). It is therefore confusing to elaborate on criteria as mentioned in line 9-26. In view of the aforementioned comments please consider rewriting this paragraph with the emphasis on risk assessment of GMOs under contained use. Annex III of Directive 2009/41/EC and the Guidance notes 2000/608/EC will be helpful.

A class of risk is assigned to GMMs or GMOs in the frame of dossiers of Belgian dossiers submitted under the Belgian Regional decrees implementing the Directive 2009/41/EC. It is possible to assign a class of risk to a GMM or GMO provided that each element used towards the achievement of the genetic modification is evaluated as well (as outlined on page 19, line 27-33). It could be noticed that one of the recommendations of the Biosafety-Europe Consortium (http://www.sciprom.ch/resources/Print-Products/Booklets/Biosafety-europe.pdf) was to merge or at least harmonise the Directives 2000/54/EC and 98/81/EC as the same control measures, based on risk assessment, can be applied to both biological agents and GMMs. But a BSL 3 organism that undergoes GM still needs to be evaluated to determine whether it is BSL3 or something else.

In view of the aforementioned comments please consider rewriting this paragraph with the emphasis on risk assessment and that similar management measures are used to minimise the risk for human health and environment, the text is adapted as follows 'the properties inherent to biological agents.'
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name and Address</th>
<th>Section 3.3 Implications of SynBio for Risk Assessment</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.</td>
<td>Carron Delphine, 'EuropaBio, <a href="mailto:d.carron@europabio.org">d.carron@europabio.org</a>,</td>
<td>- Page 16 Line 24-25: this Opinion of the scientific committees should deal with risk assessment; however, there are ample references to socio-economical, ethical and societal issues. For this reason, why would benefits to human and animal health and the environment be covered to a “limited degree”? The SCs agree this Opinion focuses on risk assessment. The SCs do not think this Opinion does more than briefly touch upon risk governance aspects.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Page 17 Line 31-32: General comment regarding “The risks to human, animal and environmental health result from the products that emerge from SynBio methods and tools”, and Page 18 Line 1-2: “... it is important to consider novel SynBio tools, methods and products and the potential risks of SynBio products”. The SC have made a selection of 6 novel developments, however the choice is contradictory to the lines above which state that it is important to consider novel tools, methods and products.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No change in the Opinion is required.</td>
<td></td>
</tr>
</tbody>
</table>
| 24.   | Lyn-Adams Ceri, 'BBSRC, ceri.lyn-adams@bbsrc.ac.uk, | - Page 18, line 13 – page 20, line 15: we agree with the content of this section. It corresponds to current gene technology instructions and safety measures. Regarding page 20, lines 7-8 the assignment of risk class should be done by the scientist according to European gene technology regulations. 
28-30: This is a very limited definition of SynBio that does not fit with currently agreed definitions or the state of the field. Specifically, it limits synthetic biology to the modification of genetic material and ignores the defining feature of synthetic biology, namely the use of engineering principles. We suggest reading Opinion I and associated discussions for a clarification of these issues. |
|       |                  | We suggest reading Opinion I and associated discussions for a clarification of these issues. |
| 25.   | Elbing Kerstin, 'German Life Science Association (V BIO e. V.), elbing@vbio.de, | Page 16, line 16 The SCs considers the assessment of risk guidance documents such as those issued by the GMO panel and/or the GMO unit of the European Food Safety Authority (EFSA). We recommend that existing risk guidance, e.g. for genetically modified organisms (GMOs) such as plants, animals and micro-organisms as well as environmental risk assessment of medicinal products should be evaluated and updated first, wherever and whenever appropriate. This process should be based on current scientific evidence. Any new risk guidance on SynBio – if necessary at all – therefore needs to refer to already existing relevant guidance, whenever possible, in order to provide a distinct European policy and legislation that is |
|       |                  | No change in the Opinion is required. |
clear for all those implementing it. We are concerned that the lack of clear indication of where the guidance is different from already existing assessments will put legislative and executive bodies on national level at risk of unintentional non-compliance.

Page 16, line 34ff We agree with the SCs that „reasonable estimations of future developments are difficult“ and support the proposal to revisit „risk assessment methodologies for SynBio at regular intervals“. For these regular reassessments as well as for the general evaluation in 10 years from now, we suggest an open process, encouraging all relevant stakeholders to contribute.

| 26. Carron Delphine, 'EuropaBio, d.carron@europabio.org, | 3.4 Risks related to SynBio Tools, Technologies and Methods | - Page 22 Fig 3: The purpose of this figure and how it would help to define risks is unclear. The figure mixes regulatory frameworks for GMO (2001/18) with technology development (continuous process) and hopes to derive tools for risk assessment. There should only be one reference point under the heading of 3.4.1., which is the risk assessment as laid down in the currently applicable regulations or directives on GMO or GMM. Our current legislation is in part product based and in part process based. With the six topics selected we see that a judgement is made based solely on the process (Synthetic Biology) if the new product would fit under the umbrella of the currently applicable GM legislation or not while disregarding the product.
In addition, the figure clearly indicates that “GM of 2014 is equal to synthetic biology of today”. This point needs to be clearer in the reminder of the text of the Opinion which often confuses current and future applications.
- Page 25 Line 37 -38: Regarding “More functional information...“, such understanding is welcomed as it stresses that with increase of knowledge and understanding the risk assessment process may also improve and uncertainty will be lowered.
- Page 27 Line 11-13: Information submitted to risk assessors especially if related to innovative technologies as synthetic biology needs to be adequately protected through Confidential Business Information provisions. Complete disclosure in the name of transparency would discourage the submissions of information to risk assessors and the registration of innovative products.
- Page 27 Line 14-19: we support the use of GMOs as comparators
- Page 28 Line 30 – 31: the paragraph that finishes with the | The aim of this Opinion is an analysis of SynBio, which obviously is a process, not a product. For clarification of the purpose of Figure 1, we refer to the figure legend and associated text. |
| 27. | Thomas Jim, 'ETC Group, jim@etcgroup.org, UK and Canada |
| 3.4 Risks related to SynBio Tools, Technologies and Methods | p24. line 35-38. The claim is made here (and implicitly and explicitly made elsewhere) that 'increasingly, detailed, precise and accurate information on the biological function of parts in genetic libraries will improve the effectiveness of risk assessment.' A dangerous assumption is being made here that should be seriously reconsidered by the committees. In effect the committee is accepting the theoretical notion of 'biological parts' as biological organisinal reality. The notion that biological organisms and systems can be divided into reducible 'parts' is a central notion undergirding the enterprise of Synthetic Biology and is a fascinating approach to attempting to construct novel working systems but it is contentious. the idea that there are in reality functional, reducible parts in ordinary real biology (as opposed to theoretical synthetic biology) is wide open to question. Its particularly questionable if biological reality as assumed here means that understanding the parts' adds up to an understanding of the whole - given the ways in which genetic sequences and elements express differently or are subject to different regulatory outcomes in different organisms, at different |

The SCs fully agree that the whole is more than the sum of the parts, in particular in SynBio, and identify the difficulties of predictive engineering in the context of biological systems as a major challenge and repeatedly refer to the challenge of assessing emerging risks due to interactions between parts in the system.

The SCs point out that the Opinion claims (implicitly and explicitly) that the whole is more than the sum of its parts. See e.g. p.39, l16-19:

"However, it is also emphasised that controlling all biological processes associated with an engineered system is not currently possible. The stochastic and probabilistic character of the underlying biochemical processes limits the drawing of a blueprint."
parts of the genome and depending on epigenetic and other factors. An interesting and relevant discussion of the limits of this 'parts' metaphor is to be found in Holdredge C, "When Engineers take Hold Of Life" In Context - a Publication of the nature Institute (2014) - see http://natureinstitute.org/pub/ic/ic32/synbio.pdf In this context relying on flawed metaphors and assumptions of predictable reducible parts could undermine the validity of risk assessment. A risk assessment that begins with an appraisal of the expected behaviour of so-called "parts" and then considers them together as if organism were actually modular and hierachical will be useless if biological reality turns out not to support the idea of organisms development being reducible in this way. There are for example indications that an organism does not simply store 'information' in a linear fashion along the DNA sequence but that the physical spatial organization of the genome within the cell also affects how information is read and transcribed and thus how organismal development proceeds. (See for example http://www.ncbi.nlm.nih.gov/pubmed/21297219 -Curr Issues Mol Biol. 2011 Feb 4;13(2):37-42. Chromosome Organization in Simple and Complex Unicellular Organisms. O'Sullivan JM.) if there is regulatory information encoded in spatial organisation then a simple risk assessment based on assessing stand-alone linear 'parts' of DNA will fail to capture expected outcomes. there may be other non-linear, non-reducible information systems within and between organisms. The point here is that those carrying out safety assessments have a responsibility to society to base their risk science on known and verifiable biological reality or organismal development, not on a hypothetical and speculative version of how biology may perhaps work or rather how biology may be reconceptualised in order to attempt to engineer it for applied purposes. The same fallacy holds on p25 line 37 where it is suggested that more functional information of parts could decrease the uncertainties of potential hazards of genetic engineering. In fact function may not be vested in parts but may emerge from the organismal level or even ecological interactions. For a useful collection of notes drawn from the literature on how a parts-based gene-driven model of organismal development and genetic regulation may not stack up to organismal reality we recommend Talbott, S "How the organism decides what to make of its Genes" - work in progress collected in at http://natureinstitute.org/txt/st/org/support/genereg.htm. In any case, when operating with biological parts, to have as much data on these parts as possible is necessary, but not sufficient to assess the risk."

In Section 3.3.3 a sentence is added clarifying that risk assessment only makes sense at the level of the biological system, not for parts in isolation. The phrases p25, l37 says: "More functional information promises to decrease the uncertainties...". However, uncertainty is the lack of certainty, hence limited knowledge and information about a system. Thus, any increase in relevant information must decrease uncertainty. The SCs did not state that all uncertainty will be gone just because some biological parts are well described.
<p>| 28. | <strong>European Federation of Biotechnology (EFB), <a href="mailto:efb@efb-central.org">efb@efb-central.org</a>,</strong> | 3.4.1 Outline of the risk assessment process | 3.4.1 Outline of the risk assessment process p. 22/page This schematic representation is confusing and is not reflecting any scientific reality. It presents the technological evolution in distinct phases, links SynBio/GMO and deduces particular reference points. Whatever purpose may have been intended, it is not clear how this can be helpful in any way. p. 23/ l. 03 Questions arising from this approach are listed, yet the initial question is never truly answered: which products should be subjected to the risk assessment? The SCs seem to accept that all products and processes in which a SynBio component is present or has been used, must be evaluated. Hence, the question on what is already covered by regulation, etc. Yet, this premise seriously overlooks that for many of the techniques now determined as constituents of SynBio no safety issues have been identified different than for similar products. In consequence, only referring to legislation on the safety of GMOs and/or pathogens provides an incomplete framework. Another example concerns cell-free systems in synthetic biology do not use living cells and would not require other safety and risk assessments than existing large-scale processes for biochemical, chemical and pharmaceutical products using enzymes. The EFB stresses that the SCs have missed an important opportunity to identify specifically those techniques that might pose new issues within so-called SynBio techniques. Instead virtually all Life Science techniques are lumped together resulting in an amalgam of potential concerns. p. 23/l. 12 Figure 4 is an unusual way of presenting the assessment process and it is not clear what this adds over similar charts presented by EFSA and others. Also the horizontal arrows in the risk assessment area (e.g. between “release”, ‘replicate’,..) are confusing and suggest a sequential order, which is incorrect. | Certainly, all products from SynBio or any other technology should be subjected to risk assessment at some level. The whole point of the Opinion is to identify which developments might pose new issues and which ones do not. It is difficult to determine where EFB identifies a “lumping together”. |
| 29. | <strong>Van der Vlugt Cecile, National Institute of Public Health and the Environment, <a href="mailto:cecile.van.der.vlugt@rivm.nl">cecile.van.der.vlugt@rivm.nl</a>,</strong> | 3.4.1 Outline of the risk assessment process | p.22 line 7: Regulatory framework for GMM’s was developed earlier, the first directives are from the nineties: 90/219/EC (contained use) and 90/220/EC (deliberate release). | P22 line 7: The SCs agree with the comment. This was the reason why line 7 explicitly reads ‘the current’ regulatory framework for GM was developed’. In order to respond to the comment, a footnote was added: ‘the first GMO Directives were the Directive 90/219/EEC on the contained use of genetically modified micro-organisms and Directive 90/220/EEC on the deliberate release of genetically modified organisms into the environment’. |</p>
<table>
<thead>
<tr>
<th>No.</th>
<th>Author</th>
<th>Section</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.</td>
<td>Westra Jaco, 'RIVM, <a href="mailto:jaco.westra@rivm.nl">jaco.westra@rivm.nl</a>',</td>
<td>3.4.1 Outline of the risk assessment process</td>
<td>P. 26/l.19-39: The working group seems to be rather optimistic in this paragraph. It reflects more hope than fact. We request that the working group provides a formulation in terms of prerequisites: e.g. in order to deal with emergent properties of complex genetically engineered organisms the following prerequisites must be fulfilled. No change in the Opinion required.</td>
</tr>
<tr>
<td>31.</td>
<td>de Lange Orlando, lmumunichwork@gm ail.com,</td>
<td>3.4.2 Risks related to SynBio developments</td>
<td>Pg. 25 ln. 5: While I agree that parts libraries that are not fully characterised will lead to people using uncharacterised components I cannot see how the activity of 'parts characterisation' can in itself lead to more people using uncharacterised components as suggested in this sentence. In addition I find it odd that 'diversity of biological functions' is put in the same sentence as that dealing with uncharacterised components. These are two real but unrelated risk factors and should be treated separately. Overall in this document there are many cases where distinct ideas or methods are unhelpfully conflated. Apart from this the suggestion to use the maximum risk level of the source organism until better data are available seems fair to me. The SCs agree with the comments and the text of the Opinion was changed as follows: p25/l5: We have changed the text to read, &quot;However, large-scale construction of SynBio libraries and use of these without detailed characterisation of individual parts, may increase the frequency of use of uncharacterised components.&quot;</td>
</tr>
</tbody>
</table>

P 22 line 26: 'these applications' has been replaced by 'GM and SynBio developments' to be in line with p22, line 19-20. P23 Figure 4: Figure 4 is meant to give an overview of important elements in the risk assessment process and is further explained in the preceding text of the sections mentioned. We have changed recommendation 4 to read,: "Support additional research and debate towards the development of sufficiently sophisticated risk assessment tools to match the advances in..."
Pg. 30, ln. 1: The Evolution from...has not yet been created’. I think achieved is meant here. There are numerous errors of grammar and spelling, bad wording and typos in this document. In most cases this did not impede reading but if I were a non-native speaker I might have found these errors more problematic, I don't think that's right for a document going out to the public of the whole EU for scrutiny. Pg. 36, ln.21: Try as I might I just can't grasp what is being suggested by 'pooling groups of genetic modifications'. I think this could be a very important point and ought to be clarified. I guess it means that if you are using CRISPR to make 3 gene knockouts at once that instead of doing 3 risk assessments you do 1. This doesn't make sense, there are 3 separate genes being mutated each with their own potential risks that are likely to be independent of the other modifications made. Have I misunderstood? Perhaps a worked example could be given. In general I think the document would really benefit from more worked examples to show how suggestions would work in practice.

5. The term ‘clearing-house’ refers to a mechanism or institution that brings together seekers and providers of goods, services or information (this matching demand and supply). With regard to biosafety information, the Biosafety Clearing house is a mechanism of the Convention on Biodiversity (CBD). Regarding the pooling of modifications, this is now clarified by giving an example. Also, instead of ‘pooling’, the SCs now use ‘categorisation’ (in terms of risks).

32. Kuiken Todd, 'Woodrow Wilson Center', todd.kuiken@wilsoncenter.org, USA

3.4.2 Risks related to SynBio developments

IV. Xenobiology Question 4, page 33, line 28: I would change "or" to "and" in the following sentence: , New variants must be tested for risk to human health or the environment and...... By having the sentence read "or" it suggests there is an option to test for just human health and not evaluate the risk to the environment.

The SCs agree and the sentence was changed accordingly.

33. Skinner Michael, 'Imperial College London and Health & Safety Executive's (HSE) Scientific Advisory Group on Genetic Modification (Contained Use) [SACGM(CU)], m.skinner@imperial.ac.uk,

3.4.2 Risks related to SynBio developments

P27, L31 With regard to Section 3.4.2 A major (and most taxing) part of the work of SACGM[CU] relates to work on GM viruses. I note that there is essentially no consideration of viruses as targets for, or subjects of, SynBio in the consultation, despite the fact that the first cited examples of genome engineering were virus applications (P34 LL 32-36).

To state (P29 L6) that “Whilst endosymbionts may not be useful as industrial organisms” totally ignores the importance of viruses (which, in many cases, can be considered as endosymbionts) to the biotechnology and biomedical sectors, especially as they are relevant to the subsequent discussion point “they may therefore offer fundamental insights into the process of genome minimisation and how the process of minimisation itself may influence risks”. Viruses, because of their size and reduced complexity compared to cellular technology assessed, to avoid an imbalance between RA and technology that might negatively impact economic and health benefits of the technology and jeopardise the quality of safety protections.”

The SCs refer to guidance explicitly addressing the risk assessment of GM viruses (p 16, line 20). According to Directive 2009/41/EC, micro-organisms are defined as any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, and animal and plant cells in culture. This sentence was modified as follows: ‘environmental risk assessment of viruses and/or medicinal products’.

Viruses are regarded as one possible field of application, and the first successful demonstrations of SynBio methodologies were in viruses (full synthesis, reverse engineering of a genome). The sections on DNA synthesis and
organisms, could well be the first viable novel organisms (in the same way that they were the first organisms recovered from synthetic DNA).

There are obvious commercial, viral applications of SynBio (notably designer phages to address antibiotic resistance), risk assessment of which is likely to prove challenging. Their omission from the Opinion document considerably weakens its contribution to the debate and to practical assessment.

34. Skinner Michael, 'Imperial College London and Health & Safety Executive's (HSE) Scientific Advisory Group on Genetic Modification (Contained Use) [SACGM(CU)], m.skinner@imperial.ac.uk,

3.4.2 Risks related to SynBio developments

P27 LL 14-19
With regard to Section 3.4.2
I applaud and fully support the recommendation concerning the benefits of using GMOs with extensive records of safe use, rather than non-GMOs, as comparators.

"Encourage the use of GMOs with proven safety records as acceptable comparators for risk assessment, i.e. the baseline state of safe organisms can advance with the complexity of new modifications. Reliance solely on non-GMO organisms, as opposed to GMOs with a history of safe use would prevent the advance of baseline risk assessment controls. In contrast, use of GMOs with a record of safety may better reflect the current understanding of risks."

No need to change the text of the Opinion.

35. European Federation of Biotechnology (EFB), efb@efb-central.org,

3.4.2 Risks related to SynBio developments

p. 24/l. 07 It is not clear if ”engineered“ in this context refers to what is defined as GMO or to SynBio. It is also not clear why a distinction is made between recombinant, mutated or synthesised DNA parts. There is no scientific basis for making a distinction between these DNA parts as they are all based on the same structure, the same information, etc. In fact, to be complete, also "wild type" DNA (if this exists) should be included, as many genetic systems will include sequences isolated from the wild type organisms. This at the same time exposes the difficulty of this reasoning: the same mutated DNA parts may be abundantly present in nature, synthetic DNA may not be different from DNA found in nature.

p. 25/l. 03 The EFB fully supports the SCs’ statement that "Research on DNA of unknown function has been conducted in molecular

p. 24/ l. 07 See definition in Opinion I: Genetic engineering in this Opinion refers in general to the techniques/methodologies used for genetic modification. Genetic material is considered to be any physical carrier of information that is inherited by offspring. The distinction in DNA-parts is merely mentioned to emphasise their presence in repositories.

No change in the Opinion required.
biology and does not present novel categories of risk.” Major collections already include safety information on the products and genetic elements that they offer.

p. 25/l. 12 (Paragraph) While there can be concerns on predicting interactions between genetic elements, it is unjustified to suggest that complexity is inherent to SynBio and that complexity will always present new challenges.

p. 26/l. 19 EFB appreciates that the SC has highlighted the issue of comparators. Firstly, there is indeed no scientific reason why GMOs that have been evaluated as safe and approved for use, should not be considered valid comparators. In fact in many sectors GMOs became the standard and excluding them as a comparator leads to a distorted representation. For certain applications of synthetic biology (e.g. development of minimal cells de novo) there may not be a close comparator. In such a case, the comparative approach may not be appropriate and a more detailed de novo characterization according to the risk classification criteria could be preferable.

p. 27/l. 11 The EFB fully supports efforts to streamline and standardise the methods for presenting genetic modification data and genetic parts information to risk assessors. These methods should be transparent and available to all stakeholders. Yet, this high level of transparency should not pre-empt the right for confidentiality to preserve the competitive nature of these developments.

p. 27/l. 20 The SC suggests that problems in risk assessment occur when there is imbalance in the sophistication of risk assessment tools and the underlying technology assessed. Given the general nature of the risk assessment paradigm that has been proposed, the EFB submits that this paradigm will in itself remain valid and not result in problems. The imbalance seems more to come from the lack of confidence in the ability to identify the specific features and hazards associated with highly complex and novel entities.

p. 29/l. 23 "minimal cells do not raise additional concerns compared to the wild type organisms they are derived from” is an incorrect statement. If the minimal organism has repressors removed compared to the wild type organism, then silent genes could be activated in the minimal organism but not the wild type. This could lead to new properties and behaviours that have gone unobserved in the wild type organism.

p.27/l.20 The SCs do not agree and maintain that further development in RA methodology is required to cope with scientific progress.

p.29/l.23: The sentence was adapted as follows: "...because minimal cells do not raise a different type of concerns compared to the wild type organisms they are derived from"

P30/l.27: The Opinion does not imply that all viable artificial cells necessarily will create the
higher than the standard risks in biological and chemistry laboratories. Given the expected performance of protocells, this shouldn’t necessarily be the case and protocells may remain crippled compared to organisms present in the environment. However, the applicability of a regulatory framework covering chemicals (such as REACH) rather than within the current GMO regulatory framework may need same high risks. This will remain to be assessed in each individual case.

| 36. | Van der Vlugt Cecile, 'National Institute of Public Health and the Environment, Cecile.van.der.Vlugt@rivm.nl, 3.4.2 Risks related to SynBio developments | p.25, line 3 'Source organism' should be replaced by 'donor organism' with respect to both GMO directives. Please check this throughout the opinion.

p.26, line 7 The risk assessment methodology leading to a containment level in order to protect human health and the environment is derived from the Directive 2009/41/EC instead of 2000/54/EC. 2000/54/EC regulates the health and safety of workers exposed to biological agents at work.

p 26, l.19 The working group addresses the aspect of 'emergence', i.e. the occurrence of new properties due to unforeseen and unpredictable (e.g. genetic) interactions as a result of using synthetic biological tools and constructs. On p. 26, line 40 the SC concludes that the current methodology of risk assessment is still appropriate. The line of reasoning by which this conclusion is reached is however unclear to us. The basic concept of 'emergence' seems not to be compatible with the notion of predictability. We request the working group to provide more insight into the line of reasoning used to arrive at this conclusion.

P25, line 3 : The SCs agree with the comment and the text was changed replacing 'source organism' by 'donor organism'

P26, line 7 : Directive 2000/54/EC involves a risk assessment (art 2 and 3) and also foresees 'containment levels'. Also, referring to the answer above, there are arguments to merge or at least harmonize the Directives 2000/54/EC and 98/81/EC as the same control measures, based on risk assessment, can be applied to both biological agents and GMMs (the Biosafety-Europe Consortium (http://www.sciprom.ch/resources/Print-Products/Booklets/Biosafety-europe.pdf ).

P26 l. 19: The reader is invited to distinguish 'methodology' and 'tools' for risk assessment. While the SCs conclude that the methodology of risk assessment (Dir 2001/18) and Dir 2009/41/EC remains appropriate, it also concludes that the application of this methodology may require novel tools, e.g. for predicting emergent properties of complex genetic systems.

Emergence does not automatically have to be unforeseeable and unpredictable. It rather means that some properties at a systemic level cannot be predicted using only information from a lower systemic level. Once the emergent characteristic has been observed and analysed it
p 26, l.33 In the discussion of the aspect of emergence, the presence of large datasets with industry is used as an argument. It is however by no means straightforward that this information can and may be available to other parties. The working group is asked to elaborate on this topic.

P.27, l.14 We support the concept of the working group that, once proven safe, a modified/synbio organism itself can serve as a comparator and as a baseline in a risk assessment process. The operationalization of this concept does not seem to be straightforward. Has the working group considered the way this can be made operational?

P.29 line 23-24 ‘... minimal cells do not raise additional concerns compared to the wild type organism.’ This is a too general conclusion, as it is obvious that exceptions will exist. What about deleting sequences which repress transcriptional regulators of silent genes evolutionary derived from a related pathogenic micro-organism?

P.30 line 25 Referring to the sentence ‘As of 2014, protocells are likely to fall within a regulatory framework covering chemicals such as REACH’, it is unclear why this is ‘as of 2014’. The cited reference does not explain the year 2014 nor the regulatory framework.

P.32, line 27-36 The presentation of a DNA sequence and an amino acid sequence in this format is very unusual and impossible to read and to understand. Please present these data in a format common to data libraries or scientific literature.

P.35, line 36-39 For risk assessment methodologies for synthetic biology the Working Group refers to the Directives 2001/18/EC and 2009/41/EC. In these directives the definition of a GMO is linked to techniques that determine if the organism will result in a a GMO or not (see annexes of the respective Directives). GMOs resulting from SynBio techniques (e.g. CRISPR/cas9 mediated by mRNA encoded cas9, line 20-23) will not always be clearly covered by the definition of a GMO. At least, member states might develop different opinions on this. If different opinions exist or arise in the future, GMOs can, in principle, be predicted in other configurations.

P.26, l.33 The SCs agree that the availability of data is not automatically guaranteed and cannot be expected.

P.27, l.14 The SCs agree that this issue it is a relevant one and it would be good to approach it in a future Opinion. The SCs will discuss this within the scope of Opinion III.

Please see the answer above.

P30 line 25: Deletion in the text of ‘as of 2014’. Pauwels et al, 2013 does not explain the regulatory framework ‘REACH’, however the paper reports on one of the conclusions of a workshop: Currently developed protocells or protocell-like systems should be considered as chemical matter rather than living organisms.

p.32, line 27-36 This format has been checked. It is a common one used in data libraries, see footnote in text: http://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi?mode=c.

P35. Line 36-39: The comment here relates to categorizing some of the Synbio applications to the techniques described in the GMO directives (annexes of the Directives). These considerations are out of the scope of the mandate of the working group. In regards the question how some of the Synbio developments relates to the definition of GM, this has been addressed in Opinion I (e.g. for
resulting from SynBio techniques might be differently assessed in EU member states. We propose to the Working Group to consider the restrictions of the GMO definition in view of future SynBio applications and to address this in their opinion. A discussion among member states on what SynBio techniques lead to a GMO and what techniques do not lead to a GMO could be helpful in order to conclude whether the GMO definition and the respective criteria have to be reconsidered to cover GMOs resulting from SynBio.

CRISPR, TALEN etc. see p 26 of Opinion I).

See the suggested edit above.

The time point of reference is the moment when this text was written.

37. Van der Vlugt Cecile, 'National Institute of Public Health and the Environment, Cecile.van.der.Vlugt @rivm.nl,

3.4.2 Risks related to SynBio developments p.35, line 25-26 'manipulation' should be 'modification' in accordance to the EU GMO directives. Please check this throughout the text. p.36, line 17 It is stated 'other aspects are discussed in section 3.4.2'. As section 3.4.2 covers page 23 to 38, it is unclear to what discussion is referred.

P35, line 25-26: The SCs agree with the comment and changed the text accordingly.

38. Carron Delphin, 'EuropaBio, d.carron@europabio.org,

3.4.2 Risks related to SynBio developments - Page 35 lines 2-4: "In contrast to the traditional transfer of genetic material from one organism to another, with minor modifications, DNA synthesis allows the generation of pervasively modified, even newly designed sequences." Edit this statement as follows: - In contrast to the traditional transfer of genetic material from one organism to another, with minor modifications, DNA synthesis can be used for both: generation of highly modified, even newly designed sequences as well as for replication of natural or slightly modified sequences.

- Page 35 lines 20-39: The paragraph refers to techniques that facilitate the introduction of genetic modifications. The techniques can be used to delete nucleotides or genes, or introduce small (single nucleotides or short sequences) or large sequences (genes). In themselves, however, these changes are not Synthetic Biology per se, and can be used to introduce similar changes as non-targeted methods. The referenced techniques can indeed also be used to introduce novel genetic elements that can be considered Synthetic Biology based on the nature of the genetic element (e.g. not found in nature). It is therefore not these techniques (CRISPR, TALENs, ZNF) but the genetic change made that should be considered.

- Page 35 lines 23-26: "These techniques may be applied in a wide range of higher organisms (plants, animals), accelerating their genetic manipulating considerably (from many months to a few weeks in the case of mice) and facilitating the manipulation of non-

The SCs do indeed not consider these techniques in isolation, but in the context of the possible genetic changes they enable.

p. 35/12-4. The suggested edit is taken over by the SCs.
model organisms.” Please edit this statement as follows: - These techniques are expected to be applied in a wide range of higher organisms in the future (plants, animals), accelerating their genetic manipulating considerably (from many months to a few weeks in the case of mice) and facilitating the modification of non-model organisms.

- Page 35 line 22 and 25: various tool “…enables the rapid introduction of targeted genetic modifications in existing genomes…”: These tools do not allow for more rapid introduction of changes in the genome compared to traditional breeding methods (transgenic, mutational or other). The tools allow for the introduction of targeted genetic modifications in existing genomes and allow for more rapid screening and characterization of the introduced change. This is key since it is commonly mentioned that these applications speed up the rate of changes, which is not the case. They allow for precise changes in the genomes and then the ability to screen for the targeted change and characterize it is made easier since the location of the change is known and not random.

- Page 35 Line 30-32: This is not clear. We suggest editing for clarity.

- Page 35 Line 32-39: we suggest not using the term “scars”. It is not accurate in a technical document and in addition mutations are natural, rampant and continuously occurring. Should we take this to mean that genomes are essentially completely composed of scar tissue?

- Page 35 line 39 references Araki, which is not in the actual list of references at the end of the document, but is a very biased single reference around the regulatory oversight of genome editing tools. This should be balanced with other references including: Hartung, F. and Schiemann, J. 2014. Precise plant breeding using new genome editing techniques: opportunities, safety and regulation in the EU. The Plant Journal (78), 742-752. Podevin, N. et. Al. 2012. Transgenic or Not? No simple answer. EMBO Reports (13), 1057-1061

p. 35/l30: the sentence is considered clear as is.

p. 35/l.32: the SCs think 'scars' are a perfectly acceptable technical term in this context. This has nothing to do with random mutations, but concerns undesired, but well defined changes to the genome.

P35, line 39, the second part of the sentence 'and there is still considerable debate whether these cases should be excluded from GMO regulations’ refers to references such as Hartung & Schiemann, and Podevin et al. The SCs deleted this part since this discussion falls outside the scope of this Opinion. Araki et al. was added to the list of references.

- Page 36 Line 3: is it true that "many" of the methods allow for multiplexed genetic modifications? This is not supported by the text on page 35 (Line 27-32) which describes only one proof-of-concept method. Suggest editing to "New methods may potentially..."

There is no doubt that many of these methods can be multiplex, and additional ones are under development. The suggested edit is therefore not appropriate. We refer to Esvelt & Wang,
<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>39.</td>
<td>Strassheim Swantje, 'German Federal Office of Consumer Protection and Food Safety, Department Genetic Engineering, <a href="mailto:swantje.strassheim@bvl.bund.de">swantje.strassheim@bvl.bund.de</a>,</td>
<td>3.4.2 Risks related to SynBio developments</td>
<td>Page 27, lines 20 to 23: This paragraph does not state a possible improvement, but names a potential problem arising from the fast development of technologies. The paragraph does not answer question 6, but could be integrated into the answer to question 5.</td>
<td>The SCs agree with the comment. See suggested revised text above (comment 35).</td>
</tr>
<tr>
<td>40.</td>
<td>Strassheim Swantje, 'German Federal Office of Consumer Protection and Food Safety, Department Genetic Engineering, <a href="mailto:swantje.strassheim@bvl.bund.de">swantje.strassheim@bvl.bund.de</a>,</td>
<td>3.4.2 Risks related to SynBio developments</td>
<td>Page 30, lines 25 to 29: This paragraph does not refer to implications for humans, animals or the environment, but to the question whether existing methods are still appropriate. It should therefore be moved to the answer to question 5.</td>
<td>The SCs agree with the comment and followed this recommendation.</td>
</tr>
<tr>
<td>41.</td>
<td>Strassheim Swantje, 'German Federal Office of Consumer Protection and Food Safety, Department Genetic Engineering, <a href="mailto:swantje.strassheim@bvl.bund.de">swantje.strassheim@bvl.bund.de</a>,</td>
<td>3.4.2 Risks related to SynBio developments</td>
<td>Page 30, lines 27 to 28: The citation &quot;Risks related to protocell research are no higher than the risks in biological and chemistry laboratories&quot; (Bedau et al., 2009) has not been cited correctly, which leads to a changed meaning. The original sentence in Bedau et al. 2009 was “…the risks entailed by protocell research today are negligible - no higher than the everyday risks in typical undergraduate biology and chemistry laboratories.” However, we suggest deleting the citation as the comparison of protocell research to an undergraduate course in biology or chemistry is not precise enough.</td>
<td>The SCs do not think that they cite Bedau incorrectly. Our assessment is derived from their argument; while we do not join their conclusion that the risks of protocell research are no higher than in undergraduate laboratories, their analysis supports our conclusion that they are no higher than in biological and chemistry laboratories (in general).</td>
</tr>
<tr>
<td>42.</td>
<td>Strassheim Swantje, 'German Federal Office of Consumer Protection and Food Safety, Department Genetic Engineering, <a href="mailto:swantje.strassheim@bvl.bund.de">swantje.strassheim@bvl.bund.de</a>,</td>
<td>3.4.2 Risks related to SynBio developments</td>
<td>Page 30, lines 35 to 39: The sentences &quot;In the future, exposure to autonomous artificial cells that survive in the laboratory and in the environment might be possible. Risk mitigation measures must be put in place to prevent these scenarios, because there are no natural reference organisms or data on interaction with other organisms and the environment” do not refer to the question since they do not relate to risk assessment but to risk management. We suggest rephrasing this part, i.e. “In the future, the exposure to autonomous artificial cells that survive in the laboratory and in the environment might be possible. Those cases would require an additional risk assessment which might be complicated if there are no natural reference organisms or data on interactions with other organisms and the environment”.</td>
<td>The SCs agree with the comment and the text was changed accordingly.</td>
</tr>
</tbody>
</table>

Old text:

"In the future, exposure to autonomous artificial cells that survive in the laboratory and in the environment might be possible. Risk mitigation measures must be put in place to prevent these scenarios, because there are no natural reference organisms or data on interaction with other organisms and the environment” do not refer to the question since they do not relate to risk assessment but to risk management."
### 3.4.2 Risks related to SynBio developments

<table>
<thead>
<tr>
<th>Page</th>
<th>Line</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>41</td>
<td>The SCs fail to see the rationale for this recommendation. However, it is agreed that the comment cited is better placed in the answer to question 6.</td>
</tr>
<tr>
<td>31</td>
<td>1 to 25</td>
<td>Page 30, lines 41 to 45 and page 31, lines 1 to 25: The two paragraphs describing “1) protocells that depend on interactions with natural cells” and “2) autonomous pro-tocells” should be integrated into the general description of protocells and not in the answer to question 5. The comment that “it is crucial to screen SynBio subfields and combinations thereof to identify unknown hazards” should be transferred to the answer to question 6.</td>
</tr>
<tr>
<td>32</td>
<td>25 to 36</td>
<td>Page 32, lines 25 to 36: The standard genetic code as it is presented in this paragraph is difficult to read. This is mainly because the letters in the five lines (AAs, Starts, Base1, Base2 and Base3) are not presented one below the other. However, even if the correct format is used, the code would be easier to read in the format as presented under <a href="http://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi?mode=t">http://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi?mode=t</a>. We therefore suggest changing the format for easier readability.</td>
</tr>
</tbody>
</table>

New text:

"In the future, the exposure to autonomous artificial cells that survive in the laboratory and in the environment might be possible. Those cases would require an additional risk assessment which might be complicated if there are no natural reference organisms or data on interactions with other organisms and the environment."

The SCs agree that this issue it is a relevant one and it would be good to approach it in a future Opinion. The SCs will discuss this within the scope of Opinion III.
Page 33, lines 18 to 22: The paragraph stating the three main aims in Xenobiology concerns all areas of Xenobiology and not only non-canonical amino acids. We suggest moving the whole paragraph to the beginning of the chapter on Xenobiology, to page 31, line 44.

Page 33, lines 26 to 31 and page 34, lines 1 to 15: The answers to the questions 4 and 5 seem somehow intermingled, although the content is appropriate. We suggest rearranging these lines as follows:

**Question 4:** The use of non-standard biochemical systems in living cells, e.g. XNA, alternative base pairs, etc., can have implications on the risk to human health or the environment. Xeno-systems could show new traits regarding evolutionary fitness, ecological competitiveness, degree of horizontal gene flow, susceptibility to viral infections, diseases or predation, toxicity. Xeno-systems could also have implications on risks as they allow for improved biocontainment, e.g. the so-called ‘genetic firewall’ that aims to avoid the exchange of genetic material through horizontal gene transfer or sexual reproduction between the genetically recoded organisms and natural organisms.

**Question 5:** Attention must be focused on determining how different forms of (semi) xenobiological organisms behave in the natural environment and if their potential for survival is lower due to their particular auxotrophy. The techniques used in XB experiments are similar to those used in genetic engineering. According to the Directives 2001/18/EC and 2009/41/EC a GMO is defined as “an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.” This definition does not specify the biochemistry of heritable material, i.e. it does not state that GMOs may only contain DNA. Existing risk assessment frameworks can legally consider xenobiological organisms, because they are considered GMOs. However, it is possible that some xenobiological products may not fall under the Directives 2001/18/EC and 2009/41/EC. For example, products of directed evolution like the incorporation of 5-chlorouracil as the 4th base (Marliere, 2011) may not be defined as a GMO with the process-based approach employed by the Directives.

P33/I18: The SCs agree. Page 33, lines 18 to 22 were moved to page 31, line 44.

P33/I26: the SCs are of the opinion that changes to answers 4 and 5 are not necessary.
<p>| 45. | Strassheim Swantje, 'German Federal Office of Consumer Protection and Food Safety, Department Genetic Engineering, swantje.strassheim @bvl.bund.de, | 3.4.2 Risks related to SynBio developments | Page 35, lines 20 to 39: New biotechnological techniques applied in plant breeding and/or the modification of other organisms have been considered in an opinion by “The New Techniques Working Group of the Commission” in 2008. Furthermore, the “Task Force on Detecting and Identifying Crops Produced with the New Plant-Breeding Techniques” has expressed in its opinion that a genetic modification must comprise at least 20 nucleotide pairs (NPs) in order to allow identification of the resulting organism based on the modification. They state that a deliberate alteration of less than 20 NPs cannot be distinguished with sufficient certainty from an incidental occurrence of this sequence. The Commission and EU countries are clarifying the legal status of these techniques (Buhk HJ: Synthetic biology and its regulation in the European Union. N Biotechnol. 2014 Dec 25;31(6):528-31.). | No changes are considered necessary. |
| 46. | Elbing Kerstin, 'German Life Science Association e. V., <a href="mailto:elbing@vbio.de">elbing@vbio.de</a>, | 3.4 Risks related to SynBio Tools, Technologies and Methods | Page 27, line 11-13: We support the idea to “streamline and standardise across EU member states the methods for submitting genetic modification data and genetic parts information to risk assessors”. A maximum of transparency is desirable. However, it has to be considered that a forced complete disclosure might discourage scientists to submit confidential business information to the risk assessors. | The SCs consider a healthy population and environment of higher importance than particular business interests. When a business plans to use organisms derived from Synbio methods/technologies, it must disclose all necessary information to the regulatory authorities in order to obtain approval. Certainly, business interests need to be protected (and appropriate measure like IPRs are in place) but not providing crucial information must not lead to a positive evaluation. |
| 47. | Thomas Jim, 'ETC Group, <a href="mailto:jim@etcgroup.org">jim@etcgroup.org</a>, UK/ Canada | 3.4.2 Risks related to SynBio developments | p27 line 14. At this and other places in the opinion the Scientific Committees propose that GMO's with &quot;proven safety records&quot; could be encouraged as acceptable comparators for risk assessment (rather than natural unmodified comparators). We would strongly urge the committees to drop this specific proposal. It is walking into a minefield of claims and counterclaims about which GMO's could be considered to have &quot;proven safety records&quot; - proven by whom? using what measures? over what period of time?, accounting for what factors? Neither the European Union or any other body vouches for 'proven safety records' of a genetically modified organism but only for provisional safety assessments at the point when they are introduced to the marketplace or the environment. Once a GMO/GMM is accepted under community processes there is no legal process to review real life impacts based on long term post release monitoring data at a later date, which would be a minimal requirement of showing a 'proven safety record'. There is currently no consensus on the inherent safety of genetically modified organisms - see Hilbeck A et al &quot;No Scientific Consensus on GMO Safety&quot; Environmental Sciences Europe 2015, 27:4 doi:10.1186/s12302-014-0034-1 and certainly strong societal disapproval of such claims. The Opinion sagely notes at page 14 the importance of 'responsible innovation', the importance of maintaining public legitimacy and support and that &quot;scientific research must not get too far ahead of public attitudes&quot;. Given the public, legal and scientific controversies attendant on determining a &quot;proven safe&quot; GMO it would be reckless to use a GMO as the unstable baseline reference point for safety of synthetically modified organisms. There are numerous organisms used for food, or other production processes (e.g. baker's yeast) that are considered safe although no one has full information of all possible interactions of each and every gene in all kinds of environment, today and for the future. However, they are considered safe. The fact that not all scientists agree that all GMOs are safe does not mean that some GMOs or organisms derived by SynBio are not safe. If every scientist would agree that all GMOs (and SynBio organisms) are safe, then we could do away altogether with risk assessment. This is clearly not what the SCs suggest. The SCs suggest that assessments should be made on a case-by-case basis and that the only difference is that engineered organisms that do have a safe track record can also be used as comparator in the risk assessment process. The issue about legitimation is certainly a valid one (who decides on what grounds), but it is not too different from the issue of who is entitled to carry out risk assessment itself - an issue that is addressed under national legislation. |
| 48. | Thomas Jim, 'ETC Group, <a href="mailto:jim@etcgroup.org">jim@etcgroup.org</a>, UK/ Canada | 3.4.2 Risks related to SynBio developments | p28-29 - lessons from endosymbionts. It may also be instructive to explore the implications of research into Megaviruses - Viruses with extremely large genomes such as the mimivirus. it has recently been suggested that these may in fact be free living organisms that lost some of their genomic material and became viruses - see &quot;Distant Mimivirus relative with a larger genome highlights the fundamental features of Megaviridae.&quot; By Defne Arslan, Matthieu Legendre, Virginie Seltzer, Chantal Abergel and Jean-Michel Claverie. PNAS, published online Oct. 10, 2011. DOI: 10.1073/pnas.1110889108. This raises the theoretical possibility that intentionally minimal genomes may also transform into viruses. This hypothesis hasn't been investigated - it is only speculative - but from a precautionary point of view its perhaps too early to make the blanket claim (as The suggestion in Arslan et al. is that Mimiviridae evolved by reductive evolution – but this does not imply that general genome reduction, without evolutionary pressure towards becoming a virus, would increase the probability of becoming a virus. If anything, it would strongly reduce this probability, as essential genes required by viruses, but not for free-living cellular survival in a bioreactor, would most likely be removed from the genome during the engineered genome minimization process. |</p>
<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Role</th>
<th>Email</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>49.</td>
<td>cat large, 'seeds, <a href="mailto:largecat@seeds.org">largecat@seeds.org</a>,</td>
<td>3.5 Opportunities for inherent safety</td>
<td>bla..bla...bla...bla...bla...</td>
<td>The SCs disregard this comment.</td>
</tr>
<tr>
<td>50.</td>
<td>Skinner Michael, 'Imperial College London and Health &amp; Safety Executive's (HSE) Scientific Advisory Group on Genetic Modification (Contained Use) [SACGM(CU)], <a href="mailto:m.skinner@imperial.ac.uk">m.skinner@imperial.ac.uk</a>,</td>
<td>3.5 Opportunities for inherent safety</td>
<td>P38 LL14-20</td>
<td>Risk in its simplest form is based on the assessment of exposure and hazard. When talking about HGT, we only talk about exposure and probability, not hazard. Current safeguards (including the one by the Church lab that was published after we opened the Opinion for public feedback) are not sufficiently low in probability to realistically out rule HGT or survival outside the lab/fermenter. This might change in the future, but the SCs stick to the original assessment.</td>
</tr>
</tbody>
</table>

With regard to Section 3.5
I note that the answer provided by the Scientific Committees to Question 7 is that currently available safety locks (auxotrophy and kill switches) are not sufficiently developed to guarantee safety. Whilst I accept that these safeguards are individually subject to the possibility of being overcome by mutation, I think that a case can be made that they may be in themselves sufficient in some situations, especially when multiple, independent auxotrophies are specified (as envisaged in Section 3.6). In particular, I would have thought that the biological containment provided by auxotrophy might be regarded as adequate when there is no foreseeable mechanism by which the GMM could cause harm to either humans or the environment. An example of this would be a situation where a safe organism, such as Bacillus subtilis, has been modified with genes that have no possibility of introducing harmful properties (e.g. a luminescence gene), and multiple auxotrophies have been introduced. After all Bacillus subtilis has been granted GRAS (Generally Regarded As Safe) status by the FDA and so it difficult to see any realistic hazard even in the unlikely event of auxotrophy being overcome as a consequence of mutation.
<table>
<thead>
<tr>
<th>Page</th>
<th>Name</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>38/l. 8</td>
<td>European Federation of Biotechnology (EFB), <a href="mailto:efb@efb-central.org">efb@efb-central.org</a>,</td>
<td>Host strains are not ‘designed to strive’ we believe this should be ‘designed to thrive’.</td>
</tr>
<tr>
<td>38/l. 32</td>
<td></td>
<td>It is indicate that “pundits” recommend developing a standardised system”. While EFB supports developing clear and science based criteria, it is strange to do this on the basis of a recommendation by undefined “pundits”.</td>
</tr>
<tr>
<td>39/l. 1</td>
<td></td>
<td>Conflicting opinions are given in these sentences leading to a very confused message.</td>
</tr>
<tr>
<td>38/l.16</td>
<td>Van der Vlugt Cecile, ‘National Institute of Public Health and the Environment, Cecile.van.der.Vlugt @rivm.nl,</td>
<td>Reliability of safety switches has only been taken into account and discussed for the situation of deliberate release. This seems to imply that safety switches are only deemed to be necessary for synbio organisms which are deliberately released. Safety switches - as an example of an inherent safety measure - could also be considered for non-deliberate/accidental release and contained use applications. In these situations this can also provide a fundamental and inherent safety mechanism. Could the working group elaborate on the aspect of ‘inherent safety’ (e.g. kill switches) for contained use as well?</td>
</tr>
</tbody>
</table>
| | | The SCs agree with the comment. Indeed, the safety mechanism should apply to both contained use and deliberate, environmental release. A safety switch that works for applications that require deliberate environmental release should in terms of reliability and strength also work for contained facilities. The text has been changed: Old text: "While genetic engineering can succeed in the design of safe organisms, currently available genetic safeguards, e.g. auxotrophy and kill switches are not reliable enough for most field releases of GMMs, because of the relative high incident of engineered bacteria escaping various genetic safeguard systems due to mutation and positive selection pressure for mutants.” New text: "Currently available genetic safeguards, e.g. auxotrophy and kill switches, however, are not reliable enough for most field releases of GMMs and accidental release of contained GMMs, because of the relative high incident of engineered bacteria escaping various genetic safeguard systems due to mutation and positive
| 53. | Strassheim Swantje, ‘German Federal Office of Consumer Protection and Food Safety, Department Genetic Engineering, swantje.strassheim @bvl.bund.de, | 3.5 Opportunities for inherent safety | Page 39, lines 2 to 4: The paragraph is about DIY biologists and their relationship to safety locks. The sentence in lines 2 to 4 reads: “When safety locks become available either generated by the academic or the DIY biologist community, DIY biologists may not use them.” However, in the next sentence it is mentioned that safer host strains could allow DIY biologists to carry out experiments in a safer environment. We suggest changing the sentence in lines 2 to 4 into “When safety locks become available either generated by the academic or the DIY biologist community, DIY biologists may use them.” | The SCs agree with the comment and the text on page 39, lines 2 to 4 was adapted: New text: “When safety locks become available either generated by the academic or the DIY biologist community, DIY biologists may, however, use them.” |
| 54. | European Federation of Biotechnology (EFB), efb@efb-central.org, | 3.6 Designing inherently safe applications | p. 39/ l. 13 The SCs appears to question whether it might be possible to avoid all adverse effects for human health and/or the environment associated to SynBio by proper design and safety engineering approaches. The EFB would appreciate that this question is reformatted or another question is added: Given the broad scope of applications/techniques that now are proposed to be considered SynBio, is it possible to present cases in which the resulting products present the same or even improved safety in comparison with similar products? The EFB submits that there are a large number of examples in diverse sectors and that imposing a legal approach similar to GMOs for such cases will present a non-justified hurdle. The question points to an unlikely hypothetical endpoint in which all safety problems have been solved. A blueprint would then identify the road towards that endpoint. When going down that road, we agree that each stop must be safer than the one that existed beforehand. No changes to the Opinion are required in relation to the comment. |
| 55. | Paton Michael, ‘Health & Safety Executive, michael.paton@hse.gsi.gov.uk, | 3.6 Designing inherently safe applications | Have the SCs considered the recent publications in the journal Nature, which demonstrate effective inherent safety mechanisms using orthologous systems – one paper describes an effective mechanism based recorded E.coli and their auxotrophic requirements for synthetic amino acids (A. J. Rovner et al. Nature http://dx.doi.org/10.1038/nature14095; 2015); and the other describes a similar organism possessing an altered genetic code that provides a resistance to evolutionary escape via mutagenesis and horizontal gene transfer ((D. J. Mandell et al. Nature http://dx.doi.org/10.1038/nature14121; 2015). Whilst the SCs opinion supports the use of orthogonal systems, these papers demonstrate a break-through in their use. The SC’S opinion should take account of these publications, to ensure that the view currently expressed is not rapidly outdated. It is important to remain flexible in the face of rapidly changing technologies and apply effective risk management strategies rather than avoidance. The SCs agree with the comment. The two papers were published after the Opinion was opened for public feedback. Certainly, these developments are taken into account when finalising the Opinion, although at the moment there is no indication that the view expressed in the Opinion is likely to be superseded soon. The two papers will be considered in the final Opinion III. |


<p>| 56. | de Lange Orlando | 3.6.1.2 Considerations for using the known containment approaches | pg. 40 In 21. I don’t think its good practice to base a very important figure (1 in a million for escapees) on a review and not the primary literature. I also have a real issue with this figure as the Moe-Behrens review referred to found that different studies had reported differences of 5 orders of magnitude in escapee percentage and some studies found no escape at all. There are massive differences in how well different methods have worked. This needs to be investigated really carefully before concluding that current safeguards are not sufficiently reliable. Not all auxotrophy or kill-switch systems are the same, some may be reliable while others may not and I expect there are logical reasons behind these differences that could be found from closer study of the literature. Also, I couldnt find Schmidt &amp; deLerenzo in the reference list. | There is absolutely no indication that current safeguard systems are sufficiently reliable. The missing reference has been added. |
| 57. | Strassheim Swantje, ‘German Federal Office of Consumer Protection and Food Safety, Department Genetic Engineering, <a href="mailto:swantje.strassheim@bvl.bund.de">swantje.strassheim@bvl.bund.de</a>, | 3.6.1.2 Considerations for using the known containment approaches | Page 40, lines 9 to 27 and page 41, lines 1 to 10: This paragraph is an enumeration of “dependent devices”. However, the bullet points do not all refer to these dependent devices. The paragraph should be changed to only three bullet points for ‘toxin-antitoxin pairs’, ‘auxotrophic organisms’ and ‘kill switches’. The remaining part of the paragraph could be streamlined to work out the conclusion that orthogonality is essential to generate inherent safety. Page 40, lines 19 to 23: Two recent articles in Nature have described the development of auxotrophic organisms (Rovner AJ et al.: Recoded organisms engineered to depend on synthetic amino acids. Nature. 2015 Jan 21. doi: 10.1038/nature14095; Mandell DJ et al.: Biocontainment of genetically modified organisms by synthetic protein design. Nature. 2015 Jan 21. doi: 10.1038/nature14121), in which the expression of several essential genes is dependent on synthetic amino acids. These cells show a much lower escape rate (escape frequencies &lt; 6.3 x 10E-12 respectively &lt; 2.2 x 10E-12) than reported in the literature until today, marking a step forward in the creation of inherent firewalls. We therefore suggest citing these new articles in the Opinion. | The text has been changed as suggested. The two Nature papers were published after the Opinion was opened for public feedback. Certainly, these developments are taken into account when finalising the Opinion, although at the moment there is no indication that the view expressed in the Opinion is likely to be superseded soon. The two papers will be considered in more detail in the final Opinion III. |
| 58. | de Lange Orlando | 4 OPINION | pg.45 In 26 Whereas earlier in the report it is stated that decisions need to be taken as to which risk procedures are appropriate for protocells from among chemistry and biology methods, yet here it is suggested that a new combined approach is needed. Why wasn’t this mentioned before. In. 39 Yes, this seems eminently reasonable, especially when combined with the comparator approach this seems like a forward thinking yet safety conscious approach. | p. 45 In 26: This suggestion was already given in Section 3.4.2. |</p>
<table>
<thead>
<tr>
<th></th>
<th>Kuiken Todd, 'Woodrow Wilson Center, <a href="mailto:todd.kuiken@wilsoncenter.org">todd.kuiken@wilsoncenter.org</a>, USA</th>
<th>4 OPINION</th>
<th>VI. Citizen Science. I would like to point you to a program that the Wilson Center in partnership with diybio.org implemented providing the DIYbio community with access to biosafety professionals and suggest mentioning this as a model to use in the future and to suggest ways to fund this moving forward. The website and functions will be updated and improved throughout 2015. <a href="http://ask.diybio.org/">http://ask.diybio.org/</a></th>
<th>No changes to the Opinion are required in relation to the comment.</th>
</tr>
</thead>
<tbody>
<tr>
<td>60.</td>
<td>European Federation of Biotechnology (EFB), <a href="mailto:efb@efb-central.org">efb@efb-central.org</a>,</td>
<td>4 OPINION</td>
<td>4 OPINION p. 43/1. 40 The sentence states that “the probability of unintentional harm might increase because DIYbio is more popular”. It is not clear what this sentence tries to convey. DIYbio is more popular than what? And how does this make harm more probable? Does the comment relate to the frequency (if more people start to perform Life Science research), to inappropriate settings (if conducted in unfit facilities), to training and awareness of non-professionals? p. 45/ l. 20 In contrast to arguments presented in the preceding text, it is now suggested that some of the techniques that create modifications without insertions might create additional challenges from a risk assessment standpoint, as they are indicated to possibly contain more pervasive changes to the genomes of living organisms than traditional genetic modification techniques. This is against the recommendation of the AHWG on New Breeding Techniques. Also it is unclear why comparisons to less predictable forms of mutagenesis are not included. The EFB suggests eliminating this reference and to focus on the resulting products. p. 45/ l. 39 The EFB supports that risk assessments focus on the characteristics of the products and not on the techniques that are used to produce them. In this respect the proper comparison for the products of gene editing techniques would be the products of different forms of mutagenesis. Only when gene editing involves insertion of sequences, would GMOs be a more appropriate comparison.</td>
<td>p. 43/1.40: The SCs agree and the text was edited as follows: “because DIYbio is now more popular than in earlier years, increasing the number of participants that could cause harm”. p. 45/1.20: The SCs disagree; there is a challenge, in that the current risk assessment procedure are in any case not consistent: they either exclude some organisms based on a process-based definition that should be included based on a product-based approach, or vice versa. No changes to the Opinion are required in relation to the comment.</td>
</tr>
<tr>
<td>61.</td>
<td>Van der Vlugt Cecile, 'National Institute of Public Health and the Environment, Cecile.van.der.Vlugt @rivm.nl,</td>
<td>4 OPINION</td>
<td>p. 41, from line 27 The analysis and assessment of the Scientific Committee is focused on the methodological aspects of risk assessment. In the Opinion, the focus is on a number of developments from the perspective of applications using an emphasis on a case-by-case approach. This Opinion has a focus on risk assessment methodologies and safety aspects for a number of technological developments in SynBio. The overall risk resulting from increased use and proliferation of synthetic biology needs to be addressed as well. The working group is asked to reflect on assessing and managing the growing use of SynBio.</td>
<td>The SCs agree with the comment. Work on this and associate Opinions of the SCs on SynBio has been motivated to a considerable extent by the observed and predicted increase in the use of SynBio; these considerations are fully taken into account in the preparation of the Opinions.</td>
</tr>
<tr>
<td>62.</td>
<td>Herrera Stephan, 'Evolva, <a href="mailto:stephanh@evolva.com">stephanh@evolva.com</a>, Swiss-based, operations in Denmark and UK</td>
<td>4 OPINION</td>
<td>As perhaps the leading European synbio venture (market cap of c. €500mn, employ c. 150 people of which c. 100 in Europe (Denmark, UK and Switzerland), overall, we think this report is reasonable and balanced. We do not agree on all the points, but we would not expect to. We do believe it is right and proper that European society has a debate on this space. As a young (10 years old) entrepreneurial company with lots to do, we find it a little hard to find the time for more detailed comments (something we would ask the committee to consider in fact as a wider constraint on entrepreneurial companies, so as to ensure that this particular segment of society is being “represented” in the review). But, we respectfully offer three key points that we hope you will consider: 1. Like many technologies, synthetic biology might seem to be many things to many people who are not familiar with it. But, for those of us who use it on a daily basis, it is just an advanced branch of biotechnology. As such, we believe that the existing biotech regulatory frameworks are the best place to start. Yes there may be gaps that need to be filled, but a logical regulatory framework is already in place, and that should be the starting point. Linked to this, one should not get carried away by the academic and media obsession with branding synbio as the next big thing in biotech (not least because this branding exercise appears to be heavily motivated by the need to secure grant money/column inches). Moreover, this branding of synbio, we believe, has needlessly done everyone a great disservice because many now incorrectly view synbio as something separate and distinct from biotech. To say that synbio is somehow separate and distinct from biotech is a bit like saying that smartphones are separate and distinct from phones. Granted, smartphones are more powerful and feature laden, but they are not themselves a qualitatively different approach. 2. Please bear in mind that synbio has potentially major benefits for both individuals and society. It allows low-impact, sustainable-</td>
<td>The SCs agree with the comment and this is reflected in detail in Opinion 1 on the definition of SynBio. No changes to the Opinion are required in relation to the comment.</td>
</tr>
</tbody>
</table>
manufacturing approaches that use less energy, land and water than current approaches. It can improve the quality, safety and healthiness of many consumer products, as well as making them more affordable. It will allow customization of products for and by individuals. It will allow currently rare or unavailable ingredients to become available. At least as we read the Preliminary Opinion, the potential benefits did not come through strongly as something to be weighed in the balance.

3. There is both a potential benefit and a risk with respect to the fact that Europe is the current world leader in speciality chemicals and food ingredients – one of the sectors that most stands to gain from synbio approaches. Europe is actually much stronger than either the USA or Asia in this space. World leading European companies include: (DK) Novozymes, Chr. Hansen; (UK): Croda, Tate & Lyle; (D): Evonik, Symrise, Henkel; (F) Roquette, Tereos; (NL) DSM, Corbion, Akzo; (B) Solvay; (IE) Kerry, Glanbia; (S) Arla; (CH) Lonza, Givaudan, Firmenich, Jungbunzlauer. This is just a small sample; there are many more. The point is that there is an enormous OPPORTUNITY for Europe to take a leadership role in the responsible adoption of such technologies. But there is also a risk that too much uncertainty in the European environment will lead to leadership passing to the USA or Asia (both regions are now making big investments in the sector, whilst Europe so far, is not).

63. Carron Delphine, ‘EuropaBio, d.carron@europabio.org,

4 OPINION

- Page 43 Line 26: remove the word “easily”, this is an exaggeration.
- Page 43 Line 27: we do not understand why the increase speed of modifications may pose challenges in risk assessment. Risk assessment begins after the product is made and it does not matter how long it takes to make that product or its speed does. A parallel can be made with chemical mutagenesis where multiple mutations are induced simultaneously at the same time (and therefore at a very high speed) without no determination of the location, number, and nature of all the mutations, let alone a concern to identify them all and individually evaluate them.

- Page 43 Line 31: change “many of the new methods allow…” to “new methods may potentially allow”

- Page 43 Line 34-38: the statement “genetic modifications introduced in parallel by large-scale DNA synthesis?” is vague and of the Opinion, but the mandate of the document required a focus on the risks, as is appropriate considering the tasks of the three SCs involved. The benefits, similar to biosecurity concerns, are largely outside the scope of the Opinion, although we have explicitly considered them wherever appropriate.
too broad. We do not understand to what it refers to. We anticipate there would be no statistically significant divergence from the host species in most tractable applications of genome editing; and if you did generate such dramatic changes, you would probably have a new species, and then you would be talking about technologies beyond the current plethora of applications of these technologies.

- Page 44: lines 4-27: With respect to the phrasing of Question 5, we believe the focus of a risk assessment for any applied biotechnology should be on the resulting product and not on the process ("activities" and "tools") used to produce it. We agree that existing risk assessment methods for GMOs and chemicals are available and applicable to the products of synthetic biology. We also agree with the notion expressed several times in this section that uncertainties presented by novel applications are best addressed by developing improved tools to collect the necessary data. Thus, there is not a need for a new risk assessment framework, but there could be a need for new input data.

- Page 44: Lines 35-37 and Page 45 Lines 26-27: These lines suggest that improvements to the risk assessment methodology are needed for the use of genetic part libraries, protocells and artificial cells. We believe the existing risk assessment paradigm, as put forward by the National Academy of Sciences in 1983 (reference: Risk Assessment in the Federal Government: Managing the Process or Red Book) represents the conventional wisdom used globally to assess risk (starting with the problem formulation) and is relevant to applications of synthetic biology. Uncertainty in the risk assessment resulting from new or novel applications is best addressed by refining the quality or quantity of the input data, which may require new tools or modelling.

- Page 45 Line 15: what does it mean "novel genetic parts"? It is not clear.

- Page 45 Line 20-23: there is no scientific basis for this assertion. In fact the state of the art is driving toward increases specificity. Off target effects can be managed and accounted for just like with traditional genetic methods and mutagenesis, backcrossing and selection against off types will deal with this. This last sentence of the paragraph should be deleted ("This might create additional

broad, and do not see what the relation is to statistics. There is no reason to assume that genome synthesis will remain limited to what is currently done. Even now, the artificial yeast genome contains major genome edits, including some that will allow rapid genome reshuffling beyond what is accessible by traditional mutagenesis.

This is a philosophical concern; a framework that is adjusted to deal with new input, is new at some level, is it not? No change to the text is needed.

A paradigm is not a methodology. The methodology can evolve, even if the paradigm remains valid, although there remains a constant need to challenge the existing paradigm and reassess its continued validity in the light of technological developments, as is done in this Opinion.

The SCs agree with the comment.

The SCs disagree with the comment, as explained above.
| 64. | Carron Delphine, 'EuropaBio, d.carron@europabio.org, | 4 OPINION | - Page 45: Lines 30-33. We disagree that acceleration of genetic modification with synthetic biology tools will challenge the risk assessment. Neither the speed nor the number of genetic modifications poses a risk in itself. The products resulting from genetic modification should be the focus of the assessment.


- Page 45 Line 41-45: the paragraph is not clear. Are you suggesting comparing new genome editing methodologies with chemical, radiation mutagenesis; if this is the case, we support. If you are suggesting comparing to transgenics, we disagree, as it should require less assessment. We also would like to re-iterate that plant NBTs do not belong to this category of genome editing methodologies. Maybe it is very important that the text is using “genome” rather than DNA editing, to stress the breadth of the changes. However this should be made very clear in the text. Lastly, ‘scanning the genomes for DNA elements of potential risk or unknown risk‘ might complicate the EU regulatory system even further. The focus on “unintended effects” should not shift towards “unknown effects”

- Pages 46 and 47: Question 7 and Question 8: These questions imply that, for synthetic biology, the only acceptable risk is zero. With no other technology do we apply a zero risk standard, and neither should it be applied for applications of synthetic biology. Advanced biotechnologies can be used in a safe manner and can also be used to provide innovative solutions to societal needs. To avoid the potential for lost opportunity resulting from unnecessary constraints, we need to apply a safety lock that is proportional to the potential risk associated with a given application. | p.45/l.30: The speed does not pose a risk, but it challenges the risk assessment procedure. That is what the text correctly states.

p.45/l.39: This would require a drastic departure from existing GM policies and the establishment of an entirely new regulatory framework. The SCs do not advocate such a drastic step due to the onset of SynBio.

p.45/l.41: The SCs fail to see what is contentious here and where the idea of comparing to radiation mutagenesis comes from. ‘Genome’ is perfectly clear to the SCs. The text says that “The initial risk evaluation of such organisms might then conducted by a scan of their genomes for DNA elements of potential risk or unknown risk, and proceed according to the risks thus identified.” The SCs discussed this again and did not agree on this paragraph. The paragraph was deleted.

P46/l47 Inherently built in safety locks refers to the idea to include a safety device “inside” the organisms and to plan and design for enhanced safety at the very start of the design process. Nowhere in the text did the SC mention zero risk. Safety measures and opportunities for benefits are already balanced, e.g. in the safety measures used in the BSL 1-4 classification. |
48

65. Westra Jaco, 'RIVM, jaco.westra@rivm.nl

4 OPINION

The working group takes as a basis the current risk assessment paradigm as is used for GMO's. The traditional risk assessment methods Could the working group elaborate whether they considered alternative possible paradigms and/or lines of reasoning? This Opinion follows the line of the traditional risk assessment. However are there other risk governing paradigms that could be used. In order to get different views it could be helpful to gathering the views of scientist not familiar with risk assessment. Has the working group considered doing this and could this be a recommendation?

Security was not in the scope of this Opinion. However, safety and security are clearly linked and when viewed together this could provide a more coherent picture. What is the Opinion of the working group on this topic?

'Long term effects' - or perhaps it's better to speak about development of the organisms - are important. Can the working group specify how they have been taken into account and how they can be investigated.

The SCs used the GMO “paradigm” as baseline, since the SCs do not see SynBio as something entirely different from GM, but rather a further development that takes up GMOs and other approaches. See Opinion I.

Although biosecurity is an important consideration in this context, it is outside the focus of this Opinion. The SCs disagree with the idea that safety and security are “clearly linked”.

66. Paton Michael, ‘Health and Safety Executive, michael.paton@hse.gsi.gov.uk,

4 OPINION

Overall the report and Opinion therein is balanced, well informed and recognises the application of existing genetic engineering risk assessment methodologies to synthetic biology applications; the recommendations seem reasonable and reflect the principles of risk management.

The SCs agree with the comment. No changes to the Opinion are required in relation to the comment.

67. Paton Michael, ‘Health & Safety Executive, michael.paton@hse.gsi.gov.uk,

4 OPINION

Uncertainty in relation to genetic parts libraries and methods – the SC Opinion recognises the importance of putting uncertainty into context and recommends streamlining and standardising data and information for risk assessment across the EU member states. Rather than placing the emphasis on Member states or the EC to do this, a better approach would be for industry or stakeholders who manage genetic parts libraries, to streamline and standardise the information they require from those generating the genetic parts before they can be deposited in the library – this would ensure a) the appropriate information is provided where possible; or b) where this is not possible, indicate the uncertainty around the specific genetic parts. It is important that this approach is not overly burdensome, (which might be the case if lead by the EC and would fail to take account of the international aspect of these libraries that extends beyond the EU) and should not delay or preclude depositing genetic parts. Rather this approach would provide the risk assessors with the relevant information needed or an indication of the uncertainty around key information, that can be considered as part

The SCs agree with the comment. No changes to the Opinion are required in relation to the comment.
<table>
<thead>
<tr>
<th>No.</th>
<th>Author and Affiliation</th>
<th>Type</th>
<th>General comments</th>
</tr>
</thead>
</table>
| 68.  | Mensik Petr, ‘ELC - Federation of European Specialty Food Ingredients Industries, elc@ecco-eu.com, | 4 OPINION | - ELC agrees with the overarching comments that the framework for risk assessment of new applications resulting from synthetic biology may be addressed using current methodology used for chemical and biological fields. Because these methodologies are based in the risk assessment paradigm put forward by NAS (Risk Assessment in the Federal Government: Managing the Process, i.e., the "Red Book"), are broadly accepted and are applicable to products from many technologies, there is no need to develop a new framework.  
- We further agree with statements in the Opinion suggesting that new or novel products resulting from practicing synthetic biology may require new input data be developed for a risk assessment. These data would reduce uncertainty associated with applications that have not been time-tested.  
- ELC would encourage a product-based, not a process-based risk assessment approach. Potential risks of importance are derived from the composition of a product, not from the process used to make it.  
- ELC would respectfully disagree that gene modification can be accelerated, as is noted many times throughout the Opinion. Tools that advance the efficiency of biological technologies are not focused on altering genes more quickly, but rather on increasing the scale of DNA synthesis and gene expression.  
- Further to that point, we do not believe that the increased advancement of modifications by new technologies will result in unforeseen and additional risk that would require new risk assessment methods. The efficiency of a process does not introduce additional risk into the resulting product.  
- ELC understands that the questions about imparting inherent safety imply that a "no risk" policy should apply to synthetic biology. A zero risk standard cannot be possibly achieved by any technology. Advanced biotechnologies can be used in a safe manner and can also be used to provide innovative solutions to societal needs. To avoid the potential for lost opportunity resulting from unnecessary constraints, we need to apply a safety lock that is proportional to the potential risk associated with a given application. |

There is no zero risk, and this is not stated in the text.
| 69. | Elbing Kerstin, 'German Life Science Association (VBIO e. V.), elbing@vbio.de, | 4 OPINION | 4 OPINION Page 45, line 39-40: We encourage a product- rather than a process-based approach since it is the characteristics of the product which determine its risk, not the techniques which led to the product. | No changes to the Opinion are required in relation to the comment. |
| 70. | Petr Mensik, 'ELC - Federation of European Specialty Food Ingredients Industries, elc@ecco-eu.com, | 4 OPINION | Page 43: Lines 10-12; 19-20; 41-42.: 1a. ELC agrees that new challenges could arise due to the nature of new products that include protocells and non-standard biological systems (e.g., XNA) and as a result of DIY citizen science. Page 43: Lines 24-28: 1b. We do not agree that the increased advancement of modifications by new technologies will result in unforeseen and additional risk that would require new risk assessment methods. The efficiency of a process does not introduce additional risk into the resulting product. Page 44: lines 4-27: 2a. With respect to the phrasing of Question 5, we believe the focus of a risk assessment for any applied biotechnology should be on the resulting product and not on the process ("activities" and "tools") used to produce it. 2b. We agree that existing risk assessment methods for GMOs and chemicals are available and applicable to the products of synthetic biology. 2c. We also agree with the notion expressed several times in this section that uncertainties presented by novel applications are best addressed by developing improved tools to collect the necessary data. Thus, there is not a need for a new risk assessment framework, but there could be a need for new input data. Page 44: Lines 35-37 and Page 45 Lines 26-27.: 3a. These lines suggest that improvements to the risk assessment methodology are needed for the use of genetic part libraries, protocells and artificial cells. We believe the existing risk assessment paradigm, as put forward by the National Academy of Sciences in 1983 (reference: Risk Assessment in the Federal Government: Managing the Process or Red Book) represents the conventional wisdom used globally to assess risk and is relevant to applications of synthetic biology. Uncertainty in the risk assessment resulting from new or novel applications is best addressed by refining the quality or quantity of the input data, which may require new tools or modelling. Page 45: Lines 30-33: 3b. We disagree that gene modification can be accelerated. Tools that advance the efficiency of biological technologies are not focused | No changes to the Opinion are required in relation to the comment. |
on altering genes more quickly, but rather on increasing the scale of DNA synthesis and gene expression. We further do not agree that advances in gene modification will challenge the case-by-case approach to risk assessment due to the extent of gene modification. The products resulting from genetic modification should be the focus of the assessment. Pages 46 and 47:

4. ELC understands that the questions about imparting inherent safety imply that a “no risk” policy should apply to synthetic biology. A zero risk standard cannot be possibly achieved by any technology. Advanced biotechnologies can be used in a safe manner and can also be used to provide innovative solutions to societal needs. To avoid the potential for lost opportunity resulting from unnecessary constraints, we need to apply a safety lock that is proportional to the potential risk associated with a given application.

71. European Federation of Biotechnology (EFB), efb@efb-central.org, 8.4 Annex IV

8.4 Annex IV p. 60 It is not clear what the purpose of this Annex is and what the intentions are. In particular the inclusion of “???” in line 5 and other unanswered questions suggests the SC has not finished compiling its information for this document.

The SCs agree with the comment. Annex IV was removed in the final version of the Opinion.
<table>
<thead>
<tr>
<th>No.</th>
<th>Name of individual/organisation</th>
<th>Comment</th>
<th>Scientific Committees Response</th>
</tr>
</thead>
</table>
| 72.7 | Axel Trefzer, PhD              | From: Trefzer, Axel [mailto:Axel.Trefzer@thermofisher.com]  
Sent: Sunday, February 08, 2015 8:13 AM  
To: SANTE C2 SCENIHR  
Subject: ThermoFisher Scientific Comments to "Preliminary Opinion on Synthetic Biology II Risk assessment methodologies and safety aspects"  
To whom it may concern,  
Apologies for sending this after deadline – due to travel issues I wasn’t able to do this earlier. We hope that despite this delay it will be possible to consider our comments and opinions in the process. In case further discussion are desired – also in preparation of the third part of the opinion – I can always be reached by email or phone.  
ThermoFisher Scientific is a leading provider of research tools in LifeSciences and with its Geneart brand has been a forerunner developing Synthetic Biology services. As such we are very interested in the ongoing process led by the European Commission to establish an Opinion on Synthetic Biology.  
In general we are in agreement with the main findings (summarized in section 4 “Opinion”) of the scientific committees described in "Preliminary Opinion on Synthetic Biology II: Risk assessment methodologies and safety aspects". Especially the statement that current methods for risk assessment are appropriate for SynBio as stated in response to question 5 of the Commission is important.  
Section 3.4.2. describes risks related to specific SynBio developments in more detail. In regards to certain statements made in this section, we’d like to comment more specifically:  
Parts and Devices: At the top of page 25 (lines 1-22 and the following pages), statements are made that working with parts of unknown function increases the risk of so-called emergent properties, which are difficult to predict.  
In our opinion it is the goal of Synthetic Biology to develop collections of well characterized parts (as outlined on page 24, lines 35-40). This will reduce the amount of DNA that is transferred to obtain a certain property to a minimum which is in stark contrast to classical genetic experiments. In | As discussed above (see comment 36)], characterizing parts do not reduce the risk of emergent properties (see suggested edits to the text above). The suggestion that the smaller amounts of DNA transferred in SynBio automatically lead to a reduced risk of emergent properties ignores the fact that the transferred sequences will be much more carefully selected, are more likely to function and interact and, therefore, will have an increased likelihood of resulting in emergent properties. |
these experiments large pieces of uncharacterized genomic DNA (frequently exceeding 100kbps and encoding >>10 genes up to full genomes) were transferred, which in our opinion has a much higher risk to result in undesired, emergent properties. Thus we conclude that Synthetic Biology will result in reduced risk to obtain emergent properties. A prerequisite for this is to invest in characterization of parts and making these well characterized parts easily available to researchers and scientists.

DNA synthesis& Genome Engineering: As described in the opinion starting on page 34, line 31 these technologies enable new approaches to direct genetic changes in living systems. We agree that these new methods result have significantly reduced the hurdles to create larger changes both by de-novo synthesis as well as direct targeted modification of genomic sequences in a living cell. It is suggested that rather than defining "genetic modification" by the method used, the result should be reviewed – i.e. how many genetic changes were introduced, irrespective whether these were introduced by modern means or breeding. We agree that this should be addressed and would be willing to contribute to such a discussion. From our position as a tools provider it is important to us that this is well regulated while at the same time these regulations should support further research and not become an impediment to technological advance.

In general we’d like to point out, that the Synthetic Biology from early days on has shown strong stewardship and a high degree of responsibility regarding risks of developments coming from the field. As an example all major DNA-synthesis companies have come together in the International Gene Synthesis Consortium (IGSC). Through this consortium common screening standards and processes were developed to ensure that any synthesis request is properly evaluated for potential biosecurity and biosafety risks. This was done voluntarily by the industry which was also recognized by US congress. Application of these standards to Parts libraries (e.g. as was done for the BioBricks library) will in itself significantly reduce the potential risk represented by these collections.

The Synthetic Biology has also from early on made efforts to engage with the general public. E.g. SynBERC, a leading Synthetic Biology initiative in the US has from its start in 2006 run a very successful public outreach program. Similar efforts have been initiated in the UK through the SynBio leadership counsel which has a practices subteam. Another example is the annual iGem competition which engages a large number of students across the globe. It reaches many undergraduates and even high school students in the early stages of their careers and developments.
Overall we appreciate the effort that is taken to assess Synthetic Biology as an emerging technology. As stated above, we are willing to contribute actively to these discussions, if appropriate. In case of questions do not hesitate to reach out.

Yours sincerely