



EUROPEAN FEDERATION OF BIOTECHNOLOGY

EFB Comments on Preliminary Opinion on Synthetic Biology II - Risk assessment methodologies and safety aspects

3 SCIENTIFIC RATIONALE

p. 11/l. 01 The following comments are of general nature, but as the system provides no other way to submit them, they are mentioned here.

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 avoid the idea 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.

- p. 11/l. 01 Please accept this comment relating to p. 05/l. 09 : "... whether existing health and environmental risk assessment practices of the European Union for Genetically Modified Organisms (GMOs) are adequate for SynBio." While this may have been the remit provided by the Commission, it is not clear what makes the risk assessment practices unique and/or automatically more suitable than any other risk assessment. The opinion missed an opportunity to point out the generic nature of risk assessment as clearly demonstrated by the risk assessment paradigm presented by EFSA.
- p. 11/l. 01 Please accept this comment relating to p. 05/l. 14: It is appreciated that the opinion indicates that considerations about the social, governance, ethical and security implications of SynBio are outside the scope of the current mandate. In consequence, these should only be considered as "background" and cannot be seen as thoroughly evaluated elements. EFB strongly supports the inclusion of an evaluation of how benefits can be maximized rather than a one-sided focus on potential and hypothetical risks.
- p. 11/l. 01 Please accept this comment relating to p. 05/l. 24: 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.
- p. 11/l. 01 Please accept this comment relating to p. 05/l. 26: 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.
- p. 11/l. 01 Please accept this comment relating to p. 05/l. 44: There seems to be confusion between risk assessment methodology and the elements that must be taken into account. The EFB submits that the risk assessment paradigm that is presented is applicable to any type of risk and therefore needs no new approach. Conversely, it would indeed be relevant to see if certain techniques, now described as part of SynBio, could create a biological with features that introduce new hazards.
- p. 11/l. 01 Please accept this comment relating to p. 09/l. 08: Synthetic biologists do not aim to "re-design existing principles", such phrasing is incorrect and misleading.
- p. 11/l. 01 Please accept this comment relating to p. 09/l. 11: "SynBio processes offer novel opportunities for the creation of new industries with profound economic implications for the European Union (EU) and other major economies." While novel opportunities are essential, applications of SynBio –as defined by the SCs- are already implemented and fundamental to keep the EU Knowledge based economy competitive. While big advancements and more innovations are expected, SynBio is already safely and successfully implemented across the Life Science sectors.

- p. 11/l. 01 Please accept this comment relating to p. 09/l. 19: “A precautionary approach in accordance with domestic legislation and other relevant international obligations is required to prevent the reduction or loss of biological diversity posed by organisms, components and products generated by SynBio.” This statement seems to indicate that there is agreement that SynBio will result in reduction of biological diversity. Given the broad scope of SynBio, this is surely not to be expected from every technique within the proposed scope. For instance, some of the so-called SynBio techniques have been used for some time, and there are no indications of such an impact. If the potential effect is to be evaluated, then it would be more appropriate to consider the specific features of the product, rather than the way in which it has been produced.
- p. 11/l. 01 Please accept this comment relating to p. 09/l. 23: The paragraph makes reference to the work of an Ad Hoc Working Group, mandated to advise on whether certain new breeding techniques result in products that should be considered GMO or not, according to the relevant European legislation. It is relevant to note that in this evaluation Synthetic Biology is indicated as a separate area, next to other techniques related to e.g. mutagenesis and plant breeding. In fact, this AHWG concluded that several of the presented techniques are similar to and improvements of “traditional” techniques and should be considered excluded from the GMO legislation. The EFB therefore opposes the view that SynBio may also include these techniques.

3.1 Introduction

- p. 11/l. 04 This paragraph illustrates the difficulty created by the broad scope of SynBio: by lumping everything in one approach, an inconsistent picture emerges that can only confuse, inspire fear and leaves a sense of lack of control. Line 19 and following indicate important developments (“the dawn of SynBio”) in a particular field of applications. Yet the previous paragraph confirms earlier and industry wide growing utilization of SynBio. The EFB sees a historic continuum of technologies that are using biological systems. The importance of Life Sciences and Bio-based economies is significantly growing in a world that seeks sustainable use of renewable and environmental friendly resources.
- p. 11/l. 07 “The pioneers of molecular biology and genetic engineering in the 1970s and 1980s harnessed their ability to engineer DNA to develop the first synthetic human insulin, and thereby launching an entirely new biological drugs industry, which has significantly contributed to the global economy to improve the quality of life of diabetic patients” is a sentence written to suggest that the scientists’ motivation was monetary rather than medical. This is another example of inappropriate and misleading language use.
- p. 11/l. 26 It is said that SynBio has grown to encompass a broad set of technologies, methods and concepts that expand the scope and scale of genetic modifications. This is incorrectly presenting the evolution as an objective, this scientific concept of “SynBio” does not exist (hence the difficulty to define it). Rather a large set of techniques has been explored (similar to other fields of science) and by lumping them together post-fact it creates an illusion of a massive, uncontrollable evolution. Each development should be evaluated on its own merits and possible applications, its potential risks and benefits. Along the same lines, it is incorrect to suggest that many of these technologies and methods evolved from genetic engineering. At the most one could say that they evolved in parallel and that all Life Science applications are based on our increasing understanding of genetic processes.
- p. 11/l. 30 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.
- p. 11/l. 35 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.

- p. 13/ l. 07 (and following sentences) 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.

3.2 Risk governance

- p. 13/l. 26 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 “.. 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.”

3.3 Implications of SynBio for risk assessment

- p. 16/l. 6 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/l. 26 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. 17/l. 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. 19/ l. 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 e.g. those that are used by EFSA’s Scientific Committee to determine the Qualified Presumption of Safety (QPS).

3.4 Risks related to SynBio Tools, Technologies and Methods

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/ I. 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/ I. 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.

3.4.2 Risks related to SynBio developments

- p. 24/ I. 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/ I. 03 The EFB fully supports the SCs’ statement that “Research on DNA of unknown function has been conducted in molecular 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/ I. 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/ I. 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/ I. 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/ I. 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. 30/l. 27 This sentence can be interpreted to mean that as soon as novel, viable artificial cells are created, risks 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 to be assessed on a case by case basis.
- p. 30/l. 45 The SCs introduce an important finding that novel biological functions can be designed without altering the DNA of target organisms. The EFB, its member associations and members have been engaged in cutting-edge Life Science techniques. Many of these, which in scientific meetings have been indicated as synthetic biology, do not result in changes of the genetic material of the organism. On this basis EFB submits that the definition presented in the Opinions I and II is flawed and should it become incorporated in law, will inevitably lead to wrong decisions.
- p. 31/l. 15 Potential mutations in a protocell's genetic material are provided as an example of unpredictable emergent properties. While this is truly an evolutionary question of great scientific relevance, it is incorrect to predict that this leads to unpredictable outcomes or even worse, de facto to problems. If a protocell is limited to required and desired functions, most mutations, like in natural systems, will lead to inactivation and auto-destruction. Also, mutations leading to hazardous products or overcoming specific biological barriers, can be anticipated should they be likely.
- p. 32/l. 25 Use of the terms 'so-called universal or standard genetic code' to describe genetic code is not warranted, given the discussion on alternative codes, and should be replaced with 'standard genetic code' for clarity.
- p. 32/l. 27 Schematic of genetic code engineering. This figure is copied from <http://www.nature.com/nature/journal/vaop/ncurrent/full/nature14121.html>, which is referred to as reference 15, but due to formatting issues it is not clear, as the lines for each base and amino acid are not aligned and it continues onto p33. The use of asterisks to denote stop codons is not explained, nor is the use of alternative start sites. To be informative, this figure needs to be adjusted for clarity.
- p. 34/l. 32 The SCs leading statement that 'DNA synthesis generates canonical biological systems' is misleading as it wrongly gives the impression that the production of biological systems in their entirety is the primary use for DNA synthesis. In practice most DNA synthesis performed at the current time is for the purpose of producing single coding regions for proteins, or genetic loci for the specific metabolic pathways.
- p. 35/l. 20 (Entire paragraph) Some points deserve further clarification: 1) An exact synthetic copy of a genetic sequence (whatever the size) shouldn't be considered any differently than the original. DNA is defined by its chemical components and by their sequence, and there is no scientific merit in giving different annotations to synthetic or so-called "natural" sequences. 2) Synthetic sequences with non-intended changes can be compared with naturally occurring phenomena such as recombination, rearrangements and repetitions. 3) Some of the techniques mentioned to allow directed insertion of sequences can also be applied to induce directed mutations. In this case, natural processes of cell repair are used to fix a directed mutation. It would be appropriate here to refer to the AHWG on New

Breeding techniques confirming that these techniques based on natural mutagenesis mechanisms can be excluded from the SynBio definition. 4) The number of introduced changes is not relevant as in other cases e.g. massive changes introduced by physical mutagenesis, the quantity is not deemed important. 5) Some techniques do not allow for an increase in speed in introducing changes, rather they will improve precision of changes and capacity of selection. On the one hand, this may be reason to exclude such techniques from treating them as SynBio, on the other hand they better reflect the SynBio ideal of design and redesign far better than a reliance on imprecise mutation.

- p. 35/l. 34 The wording “genomic scar” suggests that there is a healing process that results in a different than normal type of material. This is not the case and therefore the analogy is misleading.
- p. 36/l. 03 This point suggest that many of the new methods allow multiplexed genetic modifications, which affect a large number of loci at the same time. To what extend is this different from earlier techniques? i.e.. when sampling diverse sources for identifying variation within a species, likely multiple genetic differences are picked up, even if only one specific characteristic was targeted. It is not clear why this would necessitate evaluating risks individually, in particular if not all changes are known.
- p. 36/ l. 14 This is another call for more efficient procedures for risk assessment. It would be useful if the SCs could provide more information on what is meant by efficiency.
- p. 36/l. 29 It is unclear how SynBio “will likely foster citizen science” (further specified as attracting DIY biologists into a field traditionally reserved for highly trained professionals). The EFB acknowledges that the availability of methods, minimal need for equipment, reduction in cost of certain goods, and the public fascination for Life Science will attract non-professionals (similar to what has been known for other fields of technology and science). The specific risks associated with this development are related to lack of awareness, improper education, negligence of legal compliance, and challenges for governmental control. The EFB supports the SC’s opinion that they are not unique to synthetic biology; neither are they consequences of synthetic biology or Life Sciences as a whole.
- p. 37/l. 17 The assertion that DIYbio groups are reported to be at the forefront of developments in SynBio needs to be presented with some corroborative citations. Examples would include the recent high-profile glow in the dark plant project: <http://www.theguardian.com/environment/true-north/2013/jun/06/kickstarter-money-glow-in-the-dark-plants>, or the production of ‘bio-based’ clothing: <http://www.biocouture.co.uk>. In contrast to these high-profile examples of DIY-bio, traditional academic SynBio is regularly reported in the press, such as George Church’s work on biological kill-switches: <http://blogs.discovermagazine.com/d-brief/2015/01/21/kill-switch-genetically-modified-bacteria/>. Even though this aspect of the report downplays the capability of DIYbio, the phrasing of this aspect of the report implies that DIYbio is at a more advanced stage than it is.
- p. 38/l. 1 It is worth noting that peer review is required for effective risk assessment. The EFB questions as to where this peer review should originate from in DIY SynBio?

3.5 Opportunities for inherent safety

- p. 38/l. 8 Host strains are not ‘designed to thrive’ we believe this should be ‘designed to thrive’.
- p. 38/l. 32 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”.
- p. 39/l. 1 Conflicting opinions are given in these sentences leading to a very confused message.

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.

4 OPINION

- p. 43/l. 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.

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.