**template for Peer Review comments**

**Technical series on synthetic biology**

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| **Comments on the Technical Series on Synthetic Biology** | | | |
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| 8 | 13-15 | „Particularly, CRISPR-Cas technology is having impact in agriculture, especially in increasing plant yield, quality, disease resistance and herbicide resistance, breeding, and accelerated domestication.“ Consider to replace “is having” for “is expected to have” as by now there is no CRISPR-Cas-based genome edited plant available on the market worldwide. | |
| 9 | 37-39 | Tackling climate change by Synthetic Biology is a complex challenge, where virtually no projects are close to the market, and even research is limited. Here a realistic example should be identified to support the claim, as research and development to increase resilience of corals to climate change is in its infancy and mostly hypothetical (c.f. IUCN 2019 Redford “genetic frontiers…” p.91) | |
| 10 | 45 | Self-limiting insects (also p.31 line 27 ff) were developed by Oxitec and are tested in the environment. To our understanding those products are not marketed commercially but are still in testing phase. | |
| 11 | 8-10 | The message of this conclusion is unclear. I propose to replace it with: “It might be too early to be able to conclude on potential impacts associated of most SynBio applications.” | |
| 13 | 4 | The term “conventional LMOs” is not widely used and its meaning remains unclear in this context. Please consider using the term “transgenic LMOs” or “classical genetic engineering” (c.f. p. 131 l. 43). | |
| 13 | 35 | “due to the opportunities” consider adding the word “potential” before opportunities, as most of those applications have not proven to be beneficial at this stage. | |
| 13 | 40 | Table 1. Please consider including a definition of categories used, especially “advanced development”. | |
| 19 | 4-5 | “CRISPR-Cas technology is having impacts in agriculture, especially in increasing plant yield, quality, disease resistance and herbicide resistance, breeding and accelerated domestication (Zhu et al., 2020).” The technology can only have impacts on agriculture if products on the market have been shown to have a measurable impact. This is currently not the case for the mentioned applications. Please rephrase by e.g. “is expected to have”. | |
| 19 | 30-31 | This sentence combines two distinct observations. First, natural gene drives have been studied and second, field trials with natural gene drive have been performed. The sentence suggests that this is a heavy field of research, when combined with the list of natural gene drives in the sentence before, with extended field trials being performed. This is not the case, only few natural gene drives have been tested in the field, mostly research is attempting to observe gene drives in the wild. | |
| 19 | 42-43 | The statement is a theoretical prediction, not a fixed reality (c.f. e.g. resistance development). Suggestion is to replace “will” by “is intended to” | |
| 20 | 10 | Include “in” in “an intrinsic cellular mechanism present in almost all eukaryotic organisms“ | |
| 20 | 25 | Please consider to specify or delete “avoid food waste.” Here, it remains unclear how avoidance of food waste could be achieved by a methodology like RNAi as such. In case “avoid food waste” refers to applications such as non-browning vegetables and fruits, it might be considered that the trait is more likely modified for its aesthetic appeal to the costumer. | |
| 20 | 31-32 | Currently, it cannot be generally assessed whether off-target effects in NTOs are reduced by better design based on bioinformatic methods. This is due to the fact that for the majority of relevant NTOs, especially insects, no genome data are available or precise RNA binding parameters are unknown which otherwise could be considered in such a design. | |
| 21 | 6-15 | The entire paragraph should be revised in terms of language and parts of the content.  It should be kept in mind that knockdowns (KD) via RNAi are not generally preferable to knockouts (KO), but that both can be used for different purposes. With KD, a residual transcription may remain, but transcription might be fine-tuned. Stable KO, on the other hand, can in certain cases be established more quickly with the aid of genome editing methods than assumed here. | |
| 21 | 16-26 | Other methods such as haploid induction or reverse breeding may as well rely on RNAi-techniques (listed in Eckerstorfer et al. 2019). These could be considered to be amended in this context.  Eckerstorfer, Michael F.; Heissenberger, Andreas; Reichenbecher, Wolfram; Steinbrecher, Ricarda A.; Waßmann, Friedrich (2019): An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs). In: *Frontiers in bioengineering and biotechnology* 7, S. 319. DOI: 10.3389/fbioe.2019.00031. | |
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| 29 | 17-25 | While it is certainly true that some applications of gene drives are intended for use in “unmanged/wild settings” (see e.g. Simon et al 2018) the use of different environmental settings as categories are not unambiguously assignable for some applications, as those would affect multiple categories.  Simon, Samson; Otto, Mathias; Engelhard, Margret (2018): Synthetic gene drive: between continuity and novelty. Crucial differences between gene drive and genetically modified organisms require an adapted risk assessment for their use. In: EMBO reports (5). DOI: 10.15252/embr.201845760. | |
| 30 | 39 | “heritance” should probably read “inheritance” | |
| 30 | 27/41 | Those two headlines consider overlapping issues, as “conservation purposes” and “improving resilience of wild animal and plant populations” can subsume identical lines of research. | |
| 30 | 27/41 f. | Use of (transmissible) viruses and engineered derivatives has been discussed for biocontrol of zoonoses and vaccination purposes in wild populations (Murphy et al. 2016, Bull et al. 2018). Please consider to check the status of research of engineered viruses and eventually amend the respective chapters.  Bull, James J.; Smithson, Mark W.; Nuismer, Scott L. (2018): Transmissible Viral Vaccines. In: Trends in microbiology 26 (1), S. 6–15. DOI: 10.1016/j.tim.2017.09.007.  Murphy, Aisling A.; Redwood, Alec J.; Jarvis, Michael A. (2016): Self-disseminating vaccines for emerging infectious diseases. In: *Expert review of vaccines* 15 (1), S. 31–39. DOI: 10.1586/14760584.2016.1106942 | |
| 31 | 28 | Consider replacing “developed” by “genetically modified” | |
| 31 | 33 f. | General comment on section 3.2.2  The term “advanced development” should be precisely defined, not only for plants, but for all kinds of applications in this section. For plants, it should further be specified if applications in these sections stand for developmental/market trends or for examples for new traits. Currently, without further explanation the choice of examples such as for genome edited or classical genetically engineered plants appears arbitrary to a certain extent. Especially, in the context that a major part of plants likely to reach the market in the near future harbour traits conferring insect or herbicide resistance – such as the already marketed canola (Cibus). | |
| 31 | 36 | Yield increase can be challenging to determine robustly under relevant conditions. Please consider rephrasing “improve” by “aim to improve” | |
| 31 | 38 | Please consider to define the meaning of “advanced development” for (genome edited) farm animals to clarify the developmental status. | |
| 33 | 17-32 | HEGAAs are a relevant example for Synthetic Biology in the field of GM Viruses, but please also consider discussing the example of transgenic viruses to combat “citrus greening” as it is both relevant and comparably close to marketing. | |
| 42 | 17/18 | Comparing the aim of “suppression gene drives” and “chemical control agents” is misleading as the result can be very different depending on how it would be applied. Suppression drive might be designed to extinct a whole species, which might not be the achievable by chemical control agents. | |
| 42 | 23/24 | The choice of references might be unbalanced here and could include e.g. Critical Scientists Switzerland et al., 2019; Dolezel et al., 2020 from lines 32/33 of the same page. | |
| 42 | 31-33 | Those concerns are also raised for islands, as the escape might pose the risk of spread | |
| 42 | 42/43 | This is rather a simplification of the possible outcomes. Depending on the gene drive a construct could remain in the population for extended time frames (pulse chase dynamics) (Champer et al 2021), self-propagating suppression drives are seen as “highly invasive” (Esvelt and Gemmell 2017)  J Champer, I Kim, SE Champer, AG Clark, PW Messer (2021). Suppression gene drive in continuous space can result in unstable persistence of both drive and wild-type alleles. Molecular Ecology. 30:1086 | |
| 44 | 2-4 | This is a theoretical assumption. The synthetic gene drive could, depending on the design, have negative unintended impacts on the target population leading to population crash or even loss of species. | |
| 45 | 5-6 | This is not very precise, as the depth of intervention is clearly increased with genome editing. Off target effects are not considered to be independent of the target sequence in all cases for genome editing. That means that even though a lower number of off-targets might occur, those could accumulate in sequences related to the target sequence, which could e.g. influence multiple genes belonging to the same gene family.  The comparison of genome editing and conventional plant breeding for risk assessment is not appropriate, as the number of unintended changes cannot be a proxy for risks that might be associated (Eckerstorfer et al. 2019).  Eckerstorfer, Michael F.; Heissenberger, Andreas; Reichenbecher, Wolfram; Steinbrecher, Ricarda A.; Waßmann, Friedrich (2019): An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs). In: Frontiers in bioengineering and biotechnology 7, S. 319. DOI: 10.3389/fbioe.2019.00031 | |
| 46 | 10 | “service provided”: CBD is committed to protect biodiversity as a whole. The concept of ecosystem services is inappropriate in this regard. | |
| 50 | 40 | It would lead to an “ engineered living modified gene drive mosquito” | |
| 54 | 13-25 | It should be noted here, that synbio applications for nature conservation might not achieve the intended goal, as they would interact in complex ecosystems, and also that those applications do not correct the initial problem but instead aim to mitigate the consequences (e.g. of climate change). | |
| 56 | 42-44 | “The section does not intend to be a comprehensive list or guide of issues to be considered under any specific assessment, as every potential analysis will have to be done on a case-by-case basis and in accordance with national and international regulations.”  As this sentence contains language from the Cartagena Protocol it should be amended by reminding of the precautionary approach, by including “ and taking into account the precautionary approach” after “international regulations”. | |
| 57 | 48 | The term “benefits” is inappropriate here and should be deleted, as this section is dedicated to biosafety. Relevant for the CBD is in this case the Cartagena Protocol on Biosafety which solely intends to avoid “adverse effects on the conservation and sustainable use of biological diversity” (CP Art. 1). | |
| 58 | 38-39 | The intention of this sentence is unclear. Does it refer to natural gene drives? If those are limited in time and space, which might be an artefact and does not reflect the full scope of possibilities from natural gene drives, as successful and thus inactive natural gene drives are hard to identify and study. It is unclear if and how many species went extinct by natural gene drives, also known as selfish genes or selfish elements (Giese et al. 2019).  Giese, Bernd; Frieß, Johannes L.; Barton, Nicholas H.; Messer, Philipp W.; Débarre, Florence; Schetelig, Marc F. et al. (2019): Gene Drives: Dynamics and Regulatory Matters-A Report from the Workshop "Evaluation of Spatial and Temporal Control of Gene Drives," April 4-5, 2019, Vienna. In: BioEssays : news and reviews in molecular, cellular and developmental biology, e1900151. DOI: 10.1002/bies.201900151. | |
| 59 | 15-16 | ” For example, a “risk assessment light” could be implemented for cases with minimal changes and familiarity with the particular trait or plant of use (Eckerstorfer, Dolezel, et al., 2019;Schiemann etal. “ I do not see this statement fully supported by Eckerstorfer et al 2019, as this publication rather intents to warn against assuming the safety of nGM plants without the appropriate data from a sound risk assessment. Please consider identifying a more appropriate reference. | |
| 59 | 42 | This section on animals would benefit from a view to the regulation in the US, where plants and animals are not regulated in an analogous way. The retention of transgenic DNA in genome edited cattle (Norris et al 2020) in this context is another example of why all genome edited LMOs require robust risk assessment  Norris, A.L., Lee, S.S., Greenlees, K.J. et al. Template plasmid integration in germline genome-edited cattle. Nat Biotechnol 38, 163–164 (2020). https://doi.org/10.1038/s41587-019-0394-6 | |
| 61 | 7 | Please consider to delete „if deemed to be relevant“ as issues concerning the stability of formulated RNA or the lack of reference genomes for bioinformatics off-target research are most likely of high relevance for biosafety assessment. | |
| 65 | 36-37 | It should state “potential benefits”. Also it should be made clear that this is explicitly not in the scope of CBD. | |
| 67 | 36-38 | “others emphasise the potential benefits of gene drive applications and encourage further development and continued laboratory research (Dolezel et al., 2020).” This statement is a direct quote from the report. In the way the sentence is written here, it suggests the authors of Dolezel et al. (2020) are “others” and of this opinion, which is not the case. Consider identifying a direct source for the statement or at least mark the sentence with quotation marks for good scientific practice. | |
| 67 f. |  | This section could additionally discuss which regulatory classifications may be relevant to epigenetically modified organisms. These may arise intentionally (e.g. by RdDm) or unintentionally from the application of RNAi techniques (Dalakouras and Papadopoulou, 2020).  Dalakouras, Athanasios; Papadopoulou, Kalliope K. (2020): Epigenetic Modifications: An Unexplored Facet of Exogenous RNA Application in Plants. In: *Plants (Basel, Switzerland)* 9 (6). DOI: 10.3390/plants9060673. | |
| 68 | 12 | Delete one “that” | |
| 127 | 39-41 | It should be clearly stated here that self-regulation cannot be the key to appropriate international regulation and governance of synthetic biology. This is the task of national and international regulatory bodies and not the synthetic biology community. | |
| 132 | 27 | It should state “living modified organisms containing engineered gene drives” | |
| 133 | 28 | It might better read “the current potential inability to” | |
| 134 | 29-33 | It has to be taken into account here that possible environmental risks are not side effects that could occur in parallel to a potential beneficial solution. Applications aiming at “unprecedented environmental challenges“ can only have benefits if adverse effects on the environment can be ruled out in advance. For most applications data on actual benefits and their actual (positive or negative) impact on environment is still incomplete.  Please consider to exchange “Despite its potential benefits“ for „However actual benefits are yet mostly unclear and”. In order to clearly catch its intention line 31 should also read: “significant negative impacts” | |
| 134 | 44 | “The overlaps and gaps identified in this update suggest that opportunities exist for increased coordination amongst the Convention and its Protocols, and with other relevant international treaties, processes and initiatives converging on the governance of synthetic biology.”  It should be noted that different conventions, treaties, processes and initiatives have diverging goals, which questions whether “increased coordination” is appropriate here. The CBD recognizes such differences by the category of “biodiversity-related conventions” (see e.g. CBD COP Decision 14/30) The second part of the sentence could be rephrased by including “for increased information exchange” before “with other relevant”. | |
| 135 | 13-14 | It is not the task of the CBD and its organs to promote “research and development” in general. Nonetheless, I agree that it is imperative to prepare for appropriate regulation and risk assessment of Synthetic Biology in general. I propose to delete: “for research and development, and” | |
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Please submit your comments to [secretariat@cbd.int](mailto:secretariat@cbd.int).