Annex II
Template for submitting information on additional trends and issues that were identified and prioritized by the multidisciplinary AHTEG for information gathering

Part I. Endorsement of submission

Name of Country/Organization: Third World Network

Name of CBD National Focal point/Head of Organization endorsing: Chee Yoke Ling

Signature of the CBD National Focal Point/ Head of Organization:

Date: 24 November 2023

Part II. Submission of information

In submitting information, kindly provide the following information on one or more of the 12 trends and issues in synthetic biology as follows:

1. Trend and issue in synthetic biology chosen
2. Potential positive and potential negative impacts on the three objectives of the Convention
    a. Conservation of biological diversity
    b. Sustainable use of its components
    c. Fair and equitable sharing of the benefits arising out of the utilization of genetic resources
3. Potential gaps or challenges for risk assessment, risk management and regulation, including availability of tools for detection, identification and monitoring
4. Additional relevant considerations (e.g., socioeconomic, ethical, cultural, human health, intellectual property, liability and redress, IPLCs, public engagement, among others)
5. Timeframe to commercialization or release into the environment
6. Potential linkages to the Kunming-Montreal Global Biodiversity Framework and potential contribution to other internationally relevant goals and targets

Submission of supporting documentation:

For any publication that you may want to share as part of your submission, kindly include:

1. Name of publication(s), author, date and DOI or URL link.
2. Attach in pdf format any publication you have listed above.

Topic 3. Genome-edited plants

3. Potential gaps or challenges for risk assessment, risk management and regulation, including availability of tools for detection, identification and monitoring

Regulatory discussions on genome-edited plants are ongoing at the national or regional level, and a few countries have determined that some or all genome edited plants or animals are to be excluded from biosafety legislation. Our legal and technical interpretation is that currently deployed genome editing technologies and applications, including all techniques involving CRISPR-based systems, clearly fall within the Cartagena Protocol on Biosafety's definition of a living modified organism (LMO), whether they involve inserting, deleting or editing sequences of genomes. Please see our report here: https://biosafety-info.net/new-publications/why-genome-edited-organisms-are-not-excluded-from-the-cartagena-protocol/. Furthermore, in accordance with paragraph (g) of Article 8 of the Convention, and the newly adopted Target 17 of the Kunming-Montreal Global Biodiversity Framework, Parties should establish, strengthen capacity for, and implement in all countries, biosafety measures, inter alia, to establish or maintain menas to regulate, manage or control the risks to conservaiton and sustianable use of biodiversity associated with the use and release of LMOs resulting fomr biotechnology, taking also into account the risks to human health.

There is a wealth of literature on the unintended effects of genome editing that should be taken into consideration regarding claims of precision, safety or indistinguishability from conventional varieties:

A. Reported general risks of genome editing in the context of human biomedical research:

Burgio G, Teboul L (2020). Anticipating and Identifying Collateral Damage in Genome Editing. Trends Genet. 36(12):905-914. 157 Ledford H (2020). CRISPR gene editing in human embryos wreaks chromosomal mayhem. Nature. 583(7814):17-18 158

National Academy of Medicine, National Academy of Sciences, and the Royal Society. (2020). Heritable Human Genome Editing. Washington, DC: The National Academies Press. https://doi.org/10.17226/25665 159

New York Times (2020). Crispr Gene Editing Can Cause Unwanted Changes in Human Embryos, Study Finds. October 31st 2020. Accessed 03rd January 2021 https://www.nytimes.com/2020/10/31/health/crispr-genetics-embryos.html

Sciencemag (2021). Gene therapy trials for sickle cell disease halted after two patients develop cancer. February 16th 2021. https://www.sciencemag.org/news/2021/02/ gene-therapy-trials-sickle-cell-disease-halted-after-two-patients-develop-cancer

- *Concerns are raised that such off-target genetic alterations and chromosomal rearrangements may trigger cancers in this above comment piece.*

B. Studies reporting on-target effects:

Ono R, Yashuhiko Y, Aisaki K, Kitajima S, Kanno J and Hirabayashi Y (2019). Exosome-mediated horizontal gene transfer occurs in double-strand break repair during genome editing. Communications Biology 2, 57.

Ono R, Ishii M, Fujihara Y, Kitazawa M, Usami T, Kaneko-Ishino T, Kanno J, Ikawa M and Ishino F (2015). Double strand break repair by capture of retrotransposon sequences and reverse-transcribed spliced mRNA sequences in mouse zygotes. Scientific Reports 5, 12281.

- *Ono et al., show in the above two papers that when mouse cells are edited, foreign DNA can insert itself into the target site (on-target effects). The DNA was from a variety of sources present in the cell culture medium used to grow the cells, including goat and cow DNA deriving from the animal serum used to grow mouse cells. Moreover, integrations of DNA derived from the guide RNA, which is part of the CRISPR machinery, was also detected in the 2015 paper. This finding suggests that DNA breaks can be repaired and patched up with DNA that is present, and that may include foreign DNA. This generates an unintended transgenic organism.*

Norris AL, Lee SS, Greenlees KJ, Tadesse DA, Miller MF & Lombardi HA (2020) Template plasmid integration in germline genome-edited cattle. Nat Biotechnol. 38(2), 163–164. DOI: 10.1038/s41587-019-0394-6

- *Norris et al. show detection of transgenic DNA in the 'edited' cow, including antibiotic resistance genes. This DNA was not supposed to integrate into the cow, it is part of the DNA vector that is used to introduce the genome editing machinery during the GE process, but it was found to have integrated by accident. This was not picked up by the developer, but by the FDA when they decided to check it.*

Zhang Q, Xing HL, Wang ZP, Zhang HY, Yang F, Wang XC, Chen QJ (2018). Potential high-frequency off-target mutagenesis induced by CRISPR/Cas9 in Arabidopsis and its prevention. Plant Mol Biol. 96(4-5): 445-456.

- *Zhang et al., show off-target alterations as well as accidental integration of DNA in plants (Arabidopsis).*

C. Studies reporting on off-target effects:

Kosicki M, Tomberg K and Bradley A (2018). Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. Nat Biotechnol 36, 765-771.

- *Kosicki et al., show a range of unintended off-target effects including mutations, complex rearrangements, translocations, insertions and deletions.*

Petri K, Zhang W, Ma J, Schmidts A, Lee H, Horng JE, Kim DY, Kurt IC, Clement K, Hsu JY, Pinello L, Maus MV, Joung JK, Yeh JJ. CRISPR prime editing with ribonucleoprotein complexes in zebrafish and primary human cells. Nat Biotechnol. 2022 Feb;40(2):189-193. doi: 10.1038/s41587-021-00901-y

- *Petri et al., (2022) show unintended insertions of foreign DNA into off-target locations, when using prime editing techniques, which is a form of genome editing that is touted is being more 'specific' than the already supposedly 'specific' CRISPR techniques.*

Simeonov, D.R., Brandt, A.J., Chan, A.Y. *et al.* A large CRISPR-induced bystander mutation causes immune dysregulation. *Commun Biol* **2**, 70 (2019). https://doi.org/10.1038/s42003-019-0321-x

- *Simeonov et al., show that CRISPR editing in mice led to the duplication of a genetic sequence near that target site. This resulted in an immune dysregulation in mice, showing that unintended effects can impact the edited organism. If a livestock animal also suffered from this unintended change, it may alter the immune system and thus health of the animal.*

Tuladhar R, Yeu Y, Tyler Piazza J, Tan Z, Clemenceau JR, Wu X, Barrett Q, Herbert J, Mathews DH, Kim J, Hwang TH and Lum L (2019). CRISPR-Cas9-based mutagenesis frequently provokes on-target mRNA misregulation. Nat Commun 10, 4056, doi: 10.1038/ s41467-019-12028-5

- *Tuladhar et al. showed that when genome editing was used to try to destroy a gene (SDN-1) in order to create a novel trait, the editing did not fully destroy the gene but instead was still producing gene products 50 % of the time, and these were novel 'aberrant' peptides that are not ordinarily produced. The safety of novel peptides cannot be presumed safe if they are produced in a crop plant destined for consumption or environmental release.*

Other studies showing off-target effects:

Braatz J, Harloff HJ, Mascher M, Stein N, Himmelbach A, Jung C(2017). CRISPR-Cas9 Targeted Mutagenesis Leads to Simultaneous Modification of Different Homoeologous Gene Copies in Polyploid Oilseed Rape (Brassica napus). Plant Physiol. 174(2): 935-942. 162 Anderson EM, Haupt A, Schiel JA, Chou E, Machado HB, Strezoska Ž, Lenger S,

Cho SW, Kim S, Kim Y, Kweon J, Kim HS, Bae S, Kim JS (2014). Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. Genome Res. 24(1):132-41. doi: 10.1101/gr.162339.113 164

Lawrenson T, Shorinola O, Stacey N, Li C, Ostergaard L, Patron N, Uauy C, Harwood W (2015) Induction of targeted, heritable mutations in barley and Brassica oleracea using RNA-guided Cas9 nuclease. Genome Biol 16:258. https://doi.org/10.1186/s13059-015-0826-7 167

Lee K, Zhang Y, Kleinstiver BP, Guo JA, Aryee MJ, Miller J, Malzahn A, Zarecor S, Lawrence-Dill CJ, Joung JK, Qi Y, Wang K (2019) Activities and specificities of CRISPR/Cas9 and Cas12a nucleases for targeted mutagenesis in maize. Plant Biotechnol J 17(2):362–372.

McClelland S, Birmingham A, Vermeulen A, Smith Av (2015). Systematic analysis of CRISPR-Cas9 mismatch tolerance reveals low levels of off-target activity. J Biotechnol. 211: 56-65 163

Peterson BA, Haak DC, Nishimura MT, Teixeira PJ, James SR, Dangl JL, Nimchuk ZL (2016) Genome-wide assessment of efficiency and specificity in CRISPR/Cas9 mediated multiple site targeting in Arabidopsis. PLoS ONE 11(9):e0162169. https://doi.org/10.1371/journal.pone.0162169 168

Wolt JD, Wang K, Sashital D, Lawrence-Dill CJ (2016) Achieving plant CRISPR targeting that limits off-target effects. Plant Genome 9(3):1–8. https://doi.org/10.3835/plantgenome2016.05.0047 165

Zhu C, Bortesi L, Baysal C, Twyman RM, Fischer R, Capell T, Schillberg S, Christou P (2017) Characteristics of genome editing mutations in cereal crops. Trends Plant Sci 22(1):38–52. https://doi.org/10.1016/j.tplants.2016.08.009 166

Other general references:

Agapito-Tenfen SZ, Okoli AS, Bernstein MJ, Wikmark OG and Myhr AI (2018). Revisiting Risk Governance of GM Plants: The Need to Consider New and Emerging Gene-Editing Techniques. Front Plant Sci 9, 1874. https://www.frontiersin.org/articles/10.3389/fpls.2018.01874/full

Eckerstorfer MF et al (2019). An EU perspective on biosafety considerations for plants developed by genome editing and other new genetic modification techniques (nGMs). Front. Bioeng. Biotechnol. https://doi.org/10.3389/fbioe.2019.00031

ENSSER (2019). ENSSER Statement: New genetic modification techniques and their products pose risks that need to be assessed. ENSSER. https://ensser.org/publications/2019-publications/ensser-statement-new-genetic-modification-techniques-and-their-products-pose-risks-that-need-to-be-assessed/

ENSSER (2021) Scientific critique of Leopoldina and EASAC statements on genome edited plants in the EU: https://ensser.org/wp-content/uploads/2021/04/Greens-EFA-GMO-Study-1.pdf

Kawall K (2019). New possibilities on the horizon: Genome editing makes the whole genome accessible for changes. Front Plant Sci, 10:525. *This paper describes how genome editing can generate modifications that go beyond what can be achieved with natural variation.*

Kawall (2021). The generic risks and the potential of SDN-1 applications in crop plants. Plants 10(11). https://www.mdpi.com/2223-7747/10/11/2259/html;

Kawall K et al (2020). Broadening the GMO risk assessment in the EU for genome editing technologies in agriculture. Environmental Sciences Europe 32, Article number: 106 (2020) https://enveurope.springeropen.com/articles/10.1186/s12302-020-00361-2 ;

4. Additional relevant considerations (e.g., socioeconomic, ethical, cultural, human health, intellectual property, liability and redress, IPLCs, public engagement, among others)

Genome-edited plants raise numerous concerns that do not justify their exclusion from regulations, both in terms of safety and efficacy testing, as well as pose wider socio-economic, societal and political implications. Careful assessment of the potential for genome editing to achieve some of the purported goals is warranted, considering the considerable hype and claims being made about the techniques.

Addressing the issues of claimed benefits are the following recent publications:

Ayub N, Soto G (2023). Multiple challenges in the development of commercial crops using CRISPR/Cas technology. Plant Science 335: 111809. https://www.sciencedirect.com/science/article/abs/pii/S0168945223002261.

- *This paper outlines various challenges to successfully developing edited crops, and why LMO crops to date have relied heavily on a limited number of simple traits such as herbicide tolerance and Bt traits e.g. that these traits are dominant such that they will pass down to offspring, and technically simple to achieve. Using genome editing to generate useful, complex traits to address important agronomic or societal challenges is not currently achievable. Moreover, unintended impacts such as the incorporation of transgenic DNA (including for example DNA copies of the CRISPR guide RNA) are regularly detected (see GeneWatch UK, 2022 for full list of references), challenging the rationale of excluding edited products from regulation based on any intended design to be free of transgenes.*

Khaipho-Burch M et al (2023). Genetic modification can improve crop yields — but stop overselling it. Nature 20 September 2023. https://www.nature.com/articles/d41586-023-02895-w#ref-CR5

Scientific critique of Leopoldina and EASAC statements on genome edited plants in the EU: https://ensser.org/wp-content/uploads/2021/04/Greens-EFA-GMO-Study-1.pdf

Technical limitations due to the unpredictability of the editing process, e.g. on-target effects, may also be contributing to a bottleneck in products being commercialised. Please refer to the scientific literature therein: GeneWatch (2022) On-target effects of genome editing techniques: (Un)repaired DNA damage, a hinderance to safety and development? http://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/updated-genome-editing-techniques-un-repaired-mutations-hindering-safety-and-development-fin.pdf

Topic 4. Microbiome engineering

Microbiome engineering is a rapidly growing field, assisted by the relative technical ease in engineering microbes, including with novel genome editing technologies. Projects are working on engineering microbes for ecosystem-wide applications including soil microbiomes, in addition to livestock, poultry, and insect microbiomes for paratransgenesis applications. One such new example is the development of antibody treatments to attack microbes in livestock guts to reduce methane production (Arkea Bio, n.d.). Soil microbes are already being commercialised in the US. Another example is the Audacious project aiming to modify soil microbes purportedly to address climate change (Audacious.org).

2. Potential positive and potential negative impacts on the three objectives of the Convention, and
3. Potential gaps or challenges for risk assessment, risk management and regulation, including availability of tools for detection, identification and monitoring

With some jurisdictions potentially excluding certain genome-edited organisms from biosafety legislation, these applications raise urgent considerations for the objectives of the Convention due to isks that may impact on the conservation and sustainable use of biodiversity. Additionally, certain technologies designed to engineer microbes in their native environment are already in the processes of commercialisation outside of biosafety regulations, e.g. delivery of CRISPR machinery from *E.coli* to pathogens via plasmid conjugation, being developed by Folium Science.

Genetically modifying microorganisms raises specific concerns regarding the lack of ability to control spread and high uncertainties regarding potential impacts on wider microbial and/or host communities and/or organisms. Living modified (LM) microorganisms intended for contained use have been detected in wild environments, including for example, genetically engineered antibiotic resistance genes derived from bacteria being detected in six major Chinese rivers (Chen et al., 2012), as well as LM bacteria designed to produce food enzymes being detected in food products (Deckers et al., 2022). Perhaps even more concerning is the recent detection of antibiotic resistance genes and viable LM microbes in fermented food products such as vitamins and food enzymes (D'aes et al., 2022).

Microorganisms also act in concert with other microbial species and host organisms in ways not fully understood, in symbiotic relationships with other species as well as host organisms. How perturbing complex partnerships networks within microbial communities and/or their host organisms will impact wider environmental or host organism's health is highly uncertain. Moreover, how they may evolve raises important questions for environmental and human health with regard to the potential for pathogenic variants to emerge.

Horizontal gene transfer is a major concern with microbes and is well documented. Applications to alter microbiomes of soil or animals may in turn, impact those who consume these products.

For example, the Japanese population, as a result of their diet, have intestinal microbiomes that have acquired enzymes for processing the algal carbohydrates in seaweed through horizontal gene transfer from marine bacteria (Hehemann *et al*, 2012).

The use of microbes at scale also raises another concern, as more wild organisms, on mass scale, which is especially relevant to microbes, becomes a growing area of research and application. Genetic changes by human activity can bypass the processes of evolution for their establishment and spread in nature (Heinemann et al., 2021), raising new levels of uncertainty and risk due to cumulative effects.

4. Additional relevant considerations (e.g., socioeconomic, ethical, cultural, human health, intellectual property, liability and redress, IPLCs, public engagement, among others)

Patenting of microbes and technologies such as Guided Biotics may have adverse impacts on farmer livelihoods, or other stakeholders in the food system. Genetic drift and spread of microbes may also impact on agro-ecological or organic farming systems.

The use of microbes raises urgent challenges to current mechanisms of free, prior and informed consent, considering the inability to control their persistence and spread, including within indigenous peoples and local communities' lands and territories. Microbes may be transferred across organisms, intergenerationally, and via exposure routes e.g. consumption.

5. Timeframe to commercialization or release into the environment

The first product is being advertised as set for launch in 2025. It is a poultry feed that targets *Salmonella*. The Folium Science website says that "*Regulatory approval processes have commenced for the Brazilian market for 2023 where the first stage safety assessment has approved the technology as a Non-Genetically Modified Organism*." Further target countries for commercialisation include Thailand and the US.

References

Arkea Bio. (n.d.). https://www.arkeabio.com

https://www.genengnews.com/insights/igi-wins-70-million-to-study-microbiome-engineering-for-health-climate-challenges/

Folium Science. https://foliumscience.com/products/ (accessed 14th September, 2023).

Rubin, B. E., Diamond, S., Cress, B. F., Crits-Christoph, A., Lou, Y. C., Borges, A. L., Shivram, H., He, C., Xu, M., Zhou, Z., Smith, S. J., Rovinsky, R., Smock, D. C. J., Tang, K., Owens, T. K., Krishnappa, N., Sachdeva, R., Barrangou, R., Deutschbauer, A. M., ... Doudna, J. A. (2021). Species- and site-specific genome editing in complex bacterial communities. Nature Microbiology, 7(1), 34–47. https://doi.org/10.1038/s41564-021-01014-7

Topic 7. Technical refinement of novel delivery systems and chemistries to modify organisms in the field or in nature

2. Potential positive and potential negative impacts on the three objectives of the Convention

Techniques are advancing that are increasing the potential genetic modification of organisms directly in the wild. This builds on technologies such as gene drives and RNAi topical applications. Some novel techniques that warrant further attention for their potential impacts on the objectives of the Convention include those being developed to modify bacteria, as well as those being investigated to circumvent tissue culture and transformation methods in plants that currently limit the species/elite varieties of crops that can be genome edited, for example, pollen mediated delivery of editing machinery directly to crops, (see Sirinathsinghji (2019)). Viruses are also being developed to deliver genome editing machinery to crops following leaf inoculation, resulting in heritable editing (T. Li et al., 2021; Liu et al., 2023; Ma et al., 2020). Such applications could potentially also be developed for in-field delivery. The use of LM viruses to deliver genome editing machinery or RNAi mechanisms to organisms such as insects, are also underway (e.g. HEGAA's; see Sirinathsinghji (2019).

The engineering of organisms directly in the wild raises immediate concerns for the objectives of the Convention, particularly with regard to the potential adverse effects on the conservation and sustainable use of biodiversity, as risk assessments cannot be performed on the modified organism prior to release, if at all. Releasing genome editing machinery or RNAi relies on the false premise that these techniques do not result in unintended or unanticipated effects, which is in contradiction with scientific consensus. This is particularly recognisable within the medical field where safety impacts are of prime concern and rightly acknowledged (e.g. see Burgio & Teboul (2020); Ledford (2020); NAS & Royal Society (2020). Genome editing is associated with a variety of genetic and cellular errors that are an inherent part of the DNA repair process, including genetic insertions, deletions, inversions, translocations, rearrangements, and wider large-scale structural genomic impacts, prompting descriptions in major journals of "chromosomal mayhem". While a huge field within medicine is researching how to improve the safety profile of genome editing, in contrast, environmental applications appear to be taking a much more laissez-faire approach with regard to potential safety implications, warranting urgent assessment under the synthetic biology processes within the CBD. This is particularly pertinent for some techniques that appear to be evading biosafety legislation and thus are not subject to robust oversight.

The rise of such techniques and the increasing sophistication of editing tools also raises another f concern, as more wild organisms, on mass scale, which is especially relevant to microbes, becomes a growing area of research and application. Genetic changes by human activity can bypass the processes of evolution for their establishment and spread in nature (Heinemann et al., 2021), raising new levels of uncertainty and risk due to cumulative effects.

Detailed example of microbial engineering: Direct delivery of genome editing systems to bacterial species

An industry platform called Guided Biotics is being developed by a private UK company Folium Science, who are working on a platform aimed at engineering bacterial species directly in the native environment e.g. animal or plant microbiomes. The technique involves the delivery of CRISPR machinery to pathogenic bacterial species. Their patents also discuss the use of phage viruses to deliver CRISPR machinery to bacterial populations.

Their first product set for commercialisation is an *E.coli* product that expresses an engineered plasmid vector carrying CRISPR machinery designed to target multiple genes in *Salmonella* pathogens. The product is being marketed as poultry feed. Further to this, their website claims that the company is expanding its platform to be used in plants, including developing country staple crops: "In July 2020, the company won an Innovate UK grant to develop a treatment for *Xanthomonas* bacteria that devastate staple crops such as cassava, rice, and soy. Folium Science is working in partnership with the John Innes Centre to develop crop protection products for use on fresh fruit and vegetables and broad acre crops. They aim to reduce crop losses and boost overall performance by promoting a productive microbiome. Folium will launch their first product, targeting Salmonella in poultry, in 2021." (Science Creates, 2021).

There is little publicly available information to perform biosafety or wider risk assessments. There appears to be no peer-reviewed publication describing the technology, warranting the use of additional information sources for technology assessment. Patent searches do, however, provide some information. For example, they describe the inclusion of antibiotic resistance genes in the plasmid, though it is not clear what marker genes are being used in the final products. Kanamycin marker genes have been shown to confer cross-resistance to two newer generation antibiotics, raising concerns that these technologies may exacerbate rather than address the rise of antibiotic resistance. Moreover, plasmids and the conjugation mechanism can be highly promiscuous, and is further dependent on the type of plasmid used, which is also information that is currently not publicly available. Horizontal gene transfer via plasmid conjugation is an intended design aim of this technology, yet there are high degrees of uncertainty around the intended and unintended impacts of this process. There is potential for recombinant plasmids to incorporate additional antibiotic resistance genes, perform unintended modifications on the target organism, or result in horizontal gene transfer to non-target organisms for example. Moreover, any unintended impacts of genome editing machinery are mediated to some extent by the environmental context, which cannot be controlled for in the open environment.

Genome editing of bacterial cell communities is also being developed by both projects funded by the US military defence under DARPA funds, which is using retrotransposons to edit (e.g. Farzadfard et al., 2021; MIT News, 2021), and a recent multi-million dollar "Audacious" project in the US, which has recently reported the development of 'species-specific editing' that "provides the first broadly applicable strategy for organism- and locus-specific genetic manipulation within a microbial community, hinting at new emergent properties of member organisms and methods for controlling microorganisms within their native environments." Moreover, this work is being envisaged for a variety of applications inclusive of "agricultural, industrial, and health-relevant microbiomes" (Rubin et al., 2021). This project is being touted as

"precision microbiome engineering" and "community editing". Suggested applications include modifying the cow gut to reduce methane transmission, as well as modifying soil and human microbiomes for agricultural improvements and addressing human disease.

The known bacterial species are thought to be less than 1% of all species that exist, as more than 99% cannot be cultivated in the laboratory by routine techniques. This highlights the uncertainties of releasing genetically engineered microorganisms or DNA into the environment, let alone machinery to engineer them without the ability to characterise and assess them prior to release, and the inability to recall the modified organisms.

4. Additional relevant considerations (e.g., socioeconomic, ethical, cultural, human health, intellectual property, liability and redress, IPLCs, public engagement, among others)

The levels of uncertainty inherent in releasing genome editing machinery raises a number of biosafety considerations that may also exert adverse socio-economic and cultural impacts as a result. For example, unintended impacts on microorganisms could have unintended impacts on human health as well as on the environment. Plasmid mediated spread of antibiotic resistance genes may undermine efforts to address the health crisis of antibiotic resistant pathogens. Unpredictable impacts are possible, considering that microorganisms act in concert with other microbial species and host organisms in ways not fully understood, in symbiotic relationships with other species as well as host organisms. How perturbing complex partnerships networks within microbial communities and/or their host organisms will impact wider environmental or host organism's health is highly uncertain.

Moreover, many of the technologies under development are patented. Any further increase in patented products may further fuel corporate concentration. Further, some technologies are potentially evading biosafety oversight if they are not falling under biosafety legislation. This raises liability and redress issues for technologies that are not easily recallable or reversible, and may easily move beyond the target area considering the rapid ability of microorganisms to spread. Intended releases for staple crops such as cassava and rice raise immediate socio-economic concerns for farmers who would shoulder the burden of unintended impacts in case of these technologies going awry; as well as food safety concerns for consumers. Evading regulations also raises consumer choice concerns, without labelling and traceability to protect organic or non-LMO cultivation systems.

Alternative solutions such as transitioning away from intensive industrialised livestock and poultry farming should also be considered within an assessment process.

There are also potential dual-use concerns that may be raised by these technologies, given the military funding streams being applied, combined with the ease with which such technologies could be released on open environments for direct engineering.

5. Timeframe to commercialization or release into the environment

Commercialisation of bacterial applications appear to be already underway. The first product is being advertised as set for launch in 2025. It is a poultry feed that targets Salmonella. The

Folium Science website says that "*Regulatory approval processes have commenced for the Brazilian market for 2023 where the first stage safety assessment has approved the technology as a Non-Genetically Modified Organism*." (Folium Science). Further target countries for commercialisation include Thailand and the US.

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MIT News (2021) New method opens the door to efficient genome writing in bacteria https://news.mit.edu/2021/genome-writing-bacteria-0805

National Academy of Medicine, National Academy of Sciences, and the Royal Society. (2020). Heritable Human Genome Editing. Washington, DC: The National Academies Press. https://doi.org/10.17226/25665

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(US Patent 10,463,049 B2) Altering microbial populations and modifying microbiota https://patents.google.com/patent/US10463049B2/en https://www.agri-tech-e.co.uk/wp-content/uploads/2020/05/Folium-Science-Investment-opportunity.pdf).

Topic 9: Transient Modification of agricultural plants, pests and pathogens using RNAi or nanomaterials

2. Potential positive and potential negative impacts on the three objectives of the Convention

RNA interference (RNAi) technologies are now being developed and commercialised as external products that can be used in various applications from pesticide sprays, to animal feed additives, to post-harvest food preservation products (Heinemann & Walker, 2019). Distinct from already commercialized LMO crops that carry transgenes that encode for RNAi molecules, synthetic RNAi molecules are being developed for direct application to organisms as a form of environmental engineering (Heinemann, 2019).

There are several biosafety implications raised by 'transient modification' technologies that warrant technology assessment due to potential impacts on the objectives of the Convention, particularly with regard to the potential adverse effects on the conservation and sustainable use of biodiversity. The process of RNAi is incompletely understood, with developers claiming that the effects of RNAi, and the traits exerted, are transient and not passed down to the next generation. However, there is well-established evidence that RNAi effects can indeed be inherited, via multiple mechanisms, with developers themselves filing patents for the offspring of organisms exposed to RNAi products. As such, exposing organisms – both the target as well as all the unknown non-target organisms – to foliar RNA sprays has been described as environmental engineering that involves, rather than the release of LMOs, the release of a product that can produce LMOs upon exposure. Such a process is uncontrolled, as well as potentially exposing entire agroecosystems.

Exemplifying evidence of transgenerational, hereditary effects of RNAi mechanisms, Abdellatef et al. (2015) reported that when the aphid *Sitobion avenae* is fed transgenic barley expressing dsRNA, target gene expression levels were downregulated in the first-generation adults and persisted for several subsequent generations. Reduced target gene expression correlated with a decline in growth, reproduction, and survival rates. Morphological and physiological aberrations such as winged adults and delayed maturation were maintained over seven aphid generations. Moreover, Khajuria *et al*. (2015) and Coleman *et al.* (2015) reported similar effects in western corn rootworm (*Diabrotica virgifera virgifera*) and the green peach aphid (*Myzus persicae*), respectively, in which oral administration of dsRNA to the parental generation resulted in the second-generation exhibiting effects induced by downregulation of the target gene. Such impacts may occur in any non-target organisms that share sequence homology within the target gene. Currently, knowledge is lacking on the genomes of non-target organisms, and how amenable they are to RNAi effects.

3. Challenges to risk assessment

RNAi is also associated with unintended off-target effects where it can silence genes other than the target, and also in non-target organisms, as has already been documented for RNAi-expressing LMO crops (Baum et al., 2007). Significant knowledge gaps remain in our ability to answer fundamental questions such as which species could be exposed, what their genome

sequences are, or how similar the genomes of non-target organisms are to those of target organisms. While some species of RNAs are well known to be unstable, double-stranded RNAs (dsRNAs) have been shown to survive mammalian digestion and may exert effects on organisms, including people, who consume them. Moreover, synthetic RNAi products are being developed to be more stable and persistent in the environment with, for example, the use of nanoparticles, in order to improve efficacy. While oral exposure is being dismissed in the agritech field, medical researchers are exploiting this exposure route as a potential avenue for delivery of therapeutics, exposing an inconsistency in knowledge between the fields and a lack of sharing of information from medical research to biodiversity spheres with relevance to biosafety.

4. Additional relevant considerations (e.g., socioeconomic, ethical, cultural, human health, intellectual property, liability and redress, IPLCs, public engagement, among others)

Their development is raising controversy over how they may be regulated, with organisms modified by RNAi technologies potentially being excluded from being defined as an LMO. Despite a lack of regulation, products appear to be heading for market, including pesticide sprays, products that confer gender bias in seafood, animal feed additives to target seafood and bee pathogens. Horizon scanning and technology assessment are therefore urgently needed to keep abreast of a technology whose commercial development has overtaken any assessment of potential risk.

Biotech companies are filing patents for RNAi pesticide products that include claims of property rights to exposed organisms and their offspring, regardless of whether the exposure was intentional (S. Huang, 2018). Such patents would make owners of RNAi sprays also the owners of exposed organisms, "potentially including entire fields of conventional crops or long- lived trees and their seeds." (Heinemann, 2019). This risks a massive expansion of property rights over nature, ever more deeply entrenching the power of biotech and agribusiness companies over the food system, farmers and biodiversity itself. The use of RNAi external products or other transient technologies raise urgent challenges to current mechanisms of free, prior and informed consent, considering the inability to control exposure, including within indigenous peoples and local communities' lands and territories. Moreover, impacts may span generations, raising further issues for how consent can be sought and obtained.

Concerns are also raised by the potential for RNAi pesticide products to drift and contaminate untreated and off-target crops and ecosystems. The onus to address this genetic pollution may fall on farmers as well as indigenous peoples, as we have seen with genetic contamination from LM crops. This also raises farmer and consumer choice concerns by jeopardizing the ability for industrial and agro-ecological farming systems to co-exist.

5. Timeframe to commercialization or release into the environment

Products are already being approved or in the process of being approved.

In the US, an RNAi product is already going through the commercialisation process: Ledprona (GreenLight Biosciences).

In Brazil, according to a 2023 OECD report, there are four products approved since 2022 from Evolutta Agro Biotecnologia Ltda, and one from Sempre Agtech Ltda (https://one.oecd.org/document/ENV/CBC/MONO(2023)29/en/pdf, pg21)

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