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March 15, 2019

Secretariat of the Convention on Biological Diversity

Dear Sir/Madam:

Pursuant to request Ref.: **SCBD/CPU/DC/MA/MW/87798** dated 1 February 2019, to submit to the Secretariat (**secretariat@cbd.int**) information and supporting documentation on the three topics referred to [below]. Herein, we provide information and references to inform the deliberation regarding the need for additional guidance on risk assessment of **LMOs containing gene drives** by the AHTEG.

A. Experience in undertaking risk assessment of living modified organisms containing engineered gene drives […] (detailing how and for which cases); or else, lack of experience in doing so;

A gene drive can be defined as any genetic mechanism that leads to inheritance in the next generation of a specific allelic variation of a gene with frequency other than that expected according to classic Mendelian inheritance for a single gene, i.e. other than 50%. To date, no LMOs containing engineered gene drives have been the subject of risk assessment for small scale or general release into the environment by any regulatory authority, and in fact very few engineered gene drives have progressed beyond the early laboratory phase of research.

Although there are not yet actual cases of risk assessment for general release of LMOs containing gene drives obtained through the techniques of modern biotechnology, there is 20+ years of experience with actual cases of risk assessment for general release of LMOs, mostly crop plant, containing other introduced characteristics. Current examples of LMOs containing engineered gene drives can be assessed for risk to the environment and biodiversity in the same way as LMOs containing other engineered traits, and according to the methodology set out in Annex III of the Cartagena Protocol. Risk assessment of LMOs containing gene drives will be *case-by-case* depending on the trait that has been engineered including the gene drive, the

organism that has been modified, and the environment where it will be introduced, and the interaction between these.

As with the methodology outlined in Annex III of the protocol, risk assessment for a general release begins with 'identification of the genotypic or phenotypic characteristics of the LMO that may have adverse effects on biodiversity'. In the case of LMOs containing engineered gene drives, the nature of the gene drive defines the 'genotypic or phenotypic' characteristics of the LMO. After this step, the risk assessment proceeds as further described in Annex III, through evaluation of 'the likelihood of those adverse effects being realized' and 'the consequence of these adverse effects being realized'; an 'estimation of overall risk posed by the LMO based on the evaluation of the likelihood and consequence'; and 'where necessary, identification of strategies to manage these risks'. It should be possible to apply these steps in the risk assessment of LMOs containing engineered gene drives, and to rely on the many years of experience and actual cases of risk assessment on living modified crops.

It might also be useful to consider that the concept of using engineered gene drives to change the genetic composition of wild populations is derived from the observation of their occurrence in nature. These naturally occurring gene drives could be a useful baseline or comparator when assessing the risk of LMOs containing engineered gene drives.

Examples of documented naturally occurring gene drives are described in the following references:

- Burt, A., and Crisanti, A. (2018). Gene Drive: Evolved and Synthetic. ACS Chemical Biology 13.

- Burt, A., and Trivers, R. (2006). Genes in Conflict: the Biology of Selfish Genetic Elements (Belknap Press of Harvard University Press).

- Conner, A.J., Jacobs, J.M.E. 2019. A natural, conditional gene drive in plants. bioRxiv preprint, online Jan. 17, 2019. http://dx.doi.org/10.1101/519884doi:

- Lindholm, A.K., Dyer, K.A., Firman, R.C., Lila Fishman, Wolfgang Forstmeier, Luke Holman, Hanna Johannesson, Ulrich Knief, Hanna Kokko, Amanda M. Larracuente, et al. (2016). The Ecology and Evolutionary Dynamics of Meiotic Drive. Trends in Ecology & Evolution 31, 315–326.

- National Academies of Sciences, Engineering, and Medicine (2016). Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values (Washington, D.C.: National Academies Press).

- Sandler, L., Hiraizumi, Y., and Sandler, I. (1959). Meiotic Drive in Natural Populations of Drosophila Melanogaster. I. the Cytogenetic Basis of Segregation-Distortion. Genetics 44, 233–50.

- Werren, J.H., Nur, U., and Wu, C. I. (1988). Selfish genetic elements. Trends in Ecology & Evolution 3, 297–302.

While there are many theoretical applications of engineered gene drives in living modified organisms, for control of insect vectors of human and animal diseases, invasive species, and agricultural pests, there are very few examples of engineered gene drives having reached the proof-of-concept stage of development. These few examples might serve as the most useful cases to consider for risk assessment, as the risk assessment will be highly case dependent, and speculation on the risks associated with applications of gene drives that are far in the future and may never be realized, or are even purely hypothetical, may not be a particularly useful exercise at this time.

Examples of the few more advanced applications of engineered gene drives in LMOs obtained using the techniques of modern biotechnology that are under development can be found in the following references:

Mosquitoes (Anopholes gambiae; Anopholes stephensi)

- Gantz, V.M., Jasinskiene, N., Taratenkova, O., Fazekas, A., Macias, V.M., Bier, E., and James, A.A. (2015). Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. Proc. Natl. Acad. Sci. USA 215, E6736–E6743.

- Hammond, A., Galizi, R., Kyrou, K., Simoni, A., Siniscalchi, C., Katsanos, D., Gribble, M., Baker, D., Marois, E., Russell, S., et al. (2016). A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. Nature Biotechnology 34, 78–83.

- Kyrou, K., Hammond, A.M., Galizi, R., Kranjc, N., Burt, A., Beaghton, A.K., Nolan, T., and Crisanti, A. (2018). A CRISPR–Cas9 gene drive targeting doublesex causes complete population suppression in caged Anopheles gambiae mosquitoes. Nature Biotechnology 36, 1062–1066.

Fruit fly (Drisophila Melanogaster)

- Gantz, V.M., and Bier, E. (2015). The mutagenic chain reaction: A method for converting heterozygous to homozygous mutations. Science 348, 442–444.

Spotted-wing Drisophila (Drisophila suzukii)

- Buchman, A., Marshall, J.M., Ostrovski, D., Yang, T., and Akbari, O.S. (2018). Synthetically engineered Medea gene drive system in the worldwide crop pest Drosophila suzukii. Proceedings of the National Academy of Sciences of the United States of America 115, 4725–4730.

Mice

- Grunwald, H.A., Gantz, V.M., Poplawski, G., Xu, X.-R.S., Bier, E., and Cooper, K.L. (2019). Super-Mendelian inheritance mediated by CRISPR–Cas9 in the female mouse germline. Nature 566, 105–109.

- Yosef, I., Edry-Botzer, L., Globus, R., Shlomovitz, I., Munitz, A., Gerlic, M., and Qimron, U. (2019). A genetic system for biasing the sex ratio in mice. BioRxiv 515064.

All of these examples are living modified organisms obtained by the techniques of modern biotechnology, as defined in the Cartagena Protocol on Biosafety, i.e., modern biotechnology is 'In vitro nucleic acid techniques, including recombinant DNA and direct injection of nucleic acid into cells or organelles'[.] We do not know of any research, early or advanced (or even envisioned) on living modified organisms containing engineered gene drives that employ something other than the techniques of modern biotechnology as defined in the Cartagena Protocol for Biosafety.

This view is held by the Ad Hoc Technical Expert Group on Synthetic Biology, which has concluded that "…organisms containing engineered gene drives, fell under the definition of LMOs as per the Cartagena Protocol."

- Convention on Biological Diversity (2017). Report of the ad hoc technical expert group on synthetic biology. Montreal, Canada, 5-8 December 2017. CBD/SYN-BIO/AHTEG/2017/1/3.

And reiterated in a recent United Nations Environment Programme Report:

- UNEP (2019). Frontiers 2018/19 Emerging Issues of Environmental Concern. United Nations Environment Programme, Nairobi.

B. Challenges experienced or foreseen in undertaking risk assessment of living modified organisms containing engineered gene drives.

Because no LMOs containing engineered gene drives have been the subject of risk assessment for small scale or general release into the environment by any regulatory authority, it is not possible to identify specific challenges based on experience with actual cases. Annex III of the Cartagena Protocol recognizes that it is not practical to generalize regarding the risks posed by LMOs (including those containing gene drive constructs), as "[r]isk assessment should be carried out on a *case-by-case* basis…" is one of the general principles described in Annex III. However, as described above, risk assessment of LMOs containing gene drive constructs should be similar to other LMOs and non-LMOs with similar properties. Given this, there are several resources that should help identify challenges that might be encountered in risk assessments of gene drive LMOs.

A list of documents that have been produced through the leadership of several international institutions, including the United Nations Development Programme (UNDP), the World Health Organization (WHO) and the Grand Challenges in Global Health (GCGH) initiative co-sponsored by the Bill & Melinda Gates Foundation are reviewed in Beech et al (2009), which provide a broad range of options for considering gene drive LMOs:

- Beech, C.J., Vasan, S.S., Quinlan, M.M., Capurro, M.L., Alphey, L., Bayard, V., Bouaré, M., McLeod, M.C., Kittayapong, P., Lavery, J.V., et al. (2009). Deployment of Innovative Genetic Vector Control Strategies: Progress on Regulatory and Biosafety Aspects, Capacity Building and Development of Best-Practice Guidance. 17, 11.

One useful publication describes an effort by a group of experts to complete the first step, identification of possible adverse effects, for a risk assessment in the case of an engineered gene drive in a mosquito vector as a malaria control measure. This paper could serve as a useful example to similarly identify harms from other cases of LMOs containing engineered gene drives, or as a starting point for further discussions on next steps in the risk assessment, that is likelihood and consequences of these adverse effects to inform risk characterization and risk mitigation.

- Okumu, F., de Andrade, P.P., Savadogo, M., James, S., Roberts, A., Quemada, H., and Singh, J.A. (2017). Results from the Workshop "Problem Formulation for the Use of Gene Drive in Mosquitoes." The American Journal of Tropical Medicine and Hygiene 96, 530–533.

Points to consider for risk assessment specifically for gene drive LMOs are also covered in this document:

- National Academies of Sciences, Engineering, and Medicine (2016). Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values (Washington, D.C.: National Academies Press).

Further guidance on best practices for development of gene drive LMO mosquitoes, including some considerations for risk assessment is explored in detail in the following:

- James, S., Collins, F.H., Welkhoff, P.A., Emerson, C., Godfray, H.C.J., Gottlieb, M., Greenwood, B., Lindsay, S.W., Mbogo, C.M., Okumu, F.O., et al. (2018). Pathway to Deployment of Gene Drive Mosquitoes as a Potential Biocontrol Tool for Elimination of Malaria in Sub-Saharan Africa: Recommendations of a Scientific Working Group. The American Journal of Tropical Medicine and Hygiene 98, 1–49.

Challenges foreseen in undertaking risk assessment with gene drives will also depend on the risk assessment methodologies and procedures used by various countries, in compliance with their relevant laws. A country preparing to consider LMOs containing gene drives would benefit from an analysis of these methodologies, as was done for the Netherlands in the example below:

- Westra, J., van der Vlugt, C.J.B., Roesink, C.H., Hogervorst, P.A.M., and Glandorf, D.C.M. (2016). Gene Drives Policy Report (National Institute for Public Health and the Environment (RIVM), Netherlands).

In this case, the analysis concluded that for the Netherlands, "[t]he current methodology for the environmental risk assessment for GMOs released into the environment is also suitable for use with organisms with a gene drive. However, in order to effectively assess the potential environmental risks, additional knowledge and information of the effects at the population level are needed. The current strategy for implementing the step-by-step principle needs to be revised in the context of organisms with a gene drive." Thus, additional information needs were identified (relevant to C) below), as well as a review of the procedures by which gene drives fit into a particular regulatory process paradigm.

There has also been consideration, in these early stages of research, of risk assessments of LMOs containing engineered gene drives intended for 'contained use' research, i.e., no release into the environment, or limited/confined release into the environment of LMOs containing gene drives. The numerous publications on this topic, mostly related to gene drive applications in mosquito, provide information that is also relevant to risk assessment for general release of LMOs containing gene drives.

Some of those publications are listed here:

- Akbari, B.O.S., Bellen, H.J., Bier, E., Simon, L., Burt, A., Church, G.M., Cook, K.R., Edwards, O.R., Esvelt, K.M., Valentino, M., et al. (2015). Safeguarding gene drive experiments in the laboratory. ScienceExpress 1–5.

- Benedict, M.Q, Austin Burt, Margareth L. Capurro, Paul DeBarro, Alfred M. Handler, Keith R. Hayes, John M. Marshall, Walter J. Tabachnick, and Zach N. Adelman (2018). Recommendations for Laboratory Containment and Management of Gene Drive Systems in Arthropods. Vector Borne & Zoonotic Diseases 18, 3–13.

- Lunshof, J.E., and Birnbaum, A. (2017). Adaptive Risk Management of Gene Drive Experiments: Biosafety, Biosecurity, and Ethics. Applied Biosafeety 22, 97–103.

- National Academies of Sciences, Engineering, and Medicine (2016). Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values (Washington, D.C.: National Academies Press).

- James, S., Collins, F.H., Welkhoff, P.A., Emerson, C., Godfray, H.C.J., Gottlieb, M., Greenwood, B., Lindsay, S.W., Mbogo, C.M., Okumu, F.O., et al. (2018). Pathway to Deployment of Gene Drive Mosquitoes as a Potential Biocontrol Tool for Elimination of Malaria in Sub-Saharan Africa: Recommendations of a Scientific Working Group. The American Journal of Tropical Medicine and Hygiene 98, 1–49.

- Westra, J., van der Vlugt, C.J.B., Roesink, C.H., Hogervorst, P.A.M., and Glandorf, D.C.M. (2016). Gene Drives Policy Report (National Institute for Public Health and the Environment (RIVM), Netherlands).

C) Specific needs (if any) to properly undertake risk assessment of living modified organisms containing engineered gene drives.

Specific needs to properly undertake risk assessment of living modified organisms containing engineered gene drives should be identified on a *case-by-case* basis, depending on the nature of the introduced gene drive, the biology of the organism, and the receiving environment. There may be cases of LMOs containing gene drives where modeling will be particularly useful to inform a risk assessment, as there are cases of other LMOs where modeling has been useful to understand the potential for a particular adverse effect to occur related to the case being assessed.

See one paper discussing modeling of gene drives:

- de Jong T.J. (2017) Gene drives do not always increase in frequency: from genetic models to risk assessment. Journal of Consumer Protection and Food Safety 12: 299-307.

Gene Drive Policy

A number of publications describe discussions of policy options at various levels and specific needs to properly regulate and conduct risk assessment on LMOs containing gene drives.

The following publications on gene drive policy are relevant:

- Australian Academy of Science. 2017, May. Discussion Paper. Synthetic Gene Drives in Australia: Implications of Emerging Technologies. www.science.org.au/gene-drives.

- Brossard, D., Belluck, P., Gould, F., and Wirz, C.D. (2019). Promises and perils of gene drives: Navigating the communication of complex, post-normal science. PNAS 201805874.

- Emerson, C., James, S., Littler, K., and Randazzo, F. (Fil) (2017). Principles for gene drive research. Science 358, 1135 LP – 1136.

- Esvelt, K.M., Smidler, A.L., Catteruccia, F., and Church, G.M. (2014). Concerning RNA-guided gene drives for the alteration of wild populations. ELife 3, 1–21.

- James, S., Collins, F.H., Welkhoff, P.A., Emerson, C., Godfray, H.C.J., Gottlieb, M., Greenwood, B., Lindsay, S.W., Mbogo, C.M., Okumu, F.O., et al. (2018). Pathway to Deployment of Gene Drive Mosquitoes as a Potential Biocontrol Tool for Elimination of Malaria in Sub-Saharan Africa: Recommendations of a Scientific Working Group. The American Journal of Tropical Medicine and Hygiene 98, 1–49.

- National Academies of Sciences, Engineering, and Medicine (2016). Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values (Washington, D.C.: National Academies Press).

- Norwegian Biotechnology Advisory Board (2017). Statement on gene drives (Norwegian Biotechnology Advisory Board).

- Oye, K. (2014). Proceed With Caution. MIT Technology Review 117, 11.

- Oye, K.A., Esvelt, K., Appleton, E., Catteruccia, F., Church, G., Lightfoot, K.S.B., Mcnamara, J., Smidler, A., and Collins, J.P. (2014). Regulating Gene Drives. Science 345, 626–628.

- Rudenko, L., Palmer, M.J., and Oye, K. (2018). Considerations for the governance of gene drive organisms. Pathogens and Global Health.

Genome-Editing Policy

There is similarly relevant literature on gene-editing which merits review as it relates to the topic of gene drives. To date, gene-editing techniques, primarily utilizing CRISPR-cas9, have been used to obtain engineered gene drives in LMOs.

- Council for Agricultural Science and Technology (CAST). 2018. Genome Editing in Agriculture: Methods, Applications, and Governance—A paper in the series on The Need for Agricultural Innovation to Sustainably Feed the World by 2050. Issue Paper 60. CAST, Ames, Iowa.

- Dronov, R. and Howard, W. 2014. Gene Editing and CRISPR. Occasional Paper Series Issue 14, September 2014. Office of the Chief Scientist, Australian Government Chief Scientist.

- Duensing, N., Sprink, T., Parrott, W.A., Fedorova, M., Lema, M.A., Wolt, J.D., and Bartsch, D. (2018). Novel Features and Considerations for ERA and Regulation of Crops Produced by Genome Editing. Frontiers in Bioengineering and Bio technology 6, 79.

- Royal Netherlands Academy of Arts and Sciences (November 2016). Genome Editing, Position Paper of the Royal Netherlands Academy of Arts and Sciences. Amsterdam, KNAW.

- Shukla-Jones, A., Friedrichs, S., and Winickoff, D. (2018). Gene editing in an international context: Scientific, economic and social issues across sectors.

- Whelan, A.I. and Lema. M.A. 2015. Regulatory framework for gene editing and other new breeding techniques (NBTs) in Argentina. GM Crops Food. 6(4):253-265. doi: 10.1080/21645698.2015.1114698

- Wolt, J.D., Wang, K., and Yang, B. (2016). The Regulatory Status of Genome-edited Crops. Plant Biotechnology Journal 14, 510–518.

Positive and Negative Environmental Impacts

Discussions of potential positive and negative environmental impacts related to organisms containing gene drives should be useful in constructing risk assessments. Some references that include these discussions are the following:

- Collins, C.M., Bonds, J.A.S., Quinlan, M.M., and Mumford, J.D. (2019). Effects of the removal or reduction in density of the malaria mosquito, Anopheles gambiae s.l ., on interacting predators and competitors in local ecosystems: Malaria mosquito effects on ecosystems. Medical and Veterinary Entomology 33, 1–15.

- HCB Scientific Committee (2017). Scientific Opinion in response to the referral of 12 October 2015 concerning use of genetically modified mosquitoes for vector control (Haut Conseil des Biotechnologies (France)).

- Lindholm, A.K., Dyer, K.A., Firman, R.C., Lila Fishman, Wolfgang Forstmeier, Luke Holman, Hanna Johannesson, Ulrich Knief, Hanna Kokko, Amanda M. Larracuente, et al. (2016). The Ecology and Evolutionary Dynamics of Meiotic Drive. Trends in Ecology & Evolution 31, 315–326.

- Min, J., Smidler, A.L., Najjar, D., and Esvelt, K.M. (2018). Harnessing gene drive. Journal of Responsible Innovation 5.

- Okumu, F., de Andrade, P.P., Savadogo, M., James, S., Roberts, A., Quemada, H., and Singh, J.A. (2017). Results from the Workshop "Problem Formulation for the Use of Gene Drive in Mosquitoes." The American Journal of Tropical Medicine and Hygiene 96, 530–533.

- Westra, J., van der Vlugt, C.J.B., Roesink, C.H., Hogervorst, P.A.M., and Glandorf, D.C.M. (2016). Gene Drives Policy Report (National Institute for Public Health and the Environment (RIVM), Netherlands).

Thank you for giving us an opportunity to submit our responses.

Signed: __ _____ Dated: March 15, 2019_____

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