Republic of Bulgaria
MINISTRY OF ENVIRONMENT AND WATER

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Dr Cristiana Paşca Palmer
Executive Secretary
Secretariat of the Convention on Biological Diversity
United Nations Environment Programme
413 Saint-Jacques Street, Suite 800
Montreal, QC, H2Y 1N9 Canada

Subject: Reply of Bulgarian Ministry of Environment and Water to **Notification 2018-103**

Reference: SCBD/CP/DC/MA/MW/87791

Dear Dr Paşca Palmer,

Please find annexed to this letter the comments of the Bulgarian Ministry of Environment and Water on the specific requests for information contained in the Notification that should facilitate the work of the AHTEG.

This submission supplements the reply to Notification 2017-103 by the European Union and its Member States but the views expressed here are only those of the Bulgarian Ministry of Environment and Water.

With the present letter Bulgarian Ministry of Environment and Water would like to nominate to participate in the Online Forum on Synthetic Biology:

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Please accept, Madam, the assurance of my highest consideration.

KRASIMIR ZHIVKOV
DEPUTY MINISTER OF ENVIRONMENT AND WATER

Annex: Bulgarian comments on Notification 2018-103

Comments on Notification 2018-103 - Bulgaria

General comments

We find the operational definition of Synthetic biology a rather broad one. We think the defining feature of synthetic biology is that it allows introduction into living systems (ecosystems, organism, cells) of functionally important components (organisms, proteins, nucleic acids, lipids, metabolites, etc.) or processes (metabolic or signal transduction pathways, gene circuits, etc.) that are do not exist in nature or are significantly different from those that exist in nature. Such view falls within the definition developed by AHTEG, but is more specific and captures key features of new technologies that have appeared in the recent years.

There is a clear difference between Synthetic biology *per se* and the enabling technologies that facilitate it, but can also be used in the traditional molecular biology and genetic engineering. Examples of synthetic biology include between others:

1. Design of biological macromolecules (proteins, DNA and RNA) with new properties – those molecules can catalyze chemical and metabolic reactions, incl. novel ones, interact with cellular compounds and change their activity, and so on. Such design can be done *de novo* using the principles of physical and quantum chemistry and/or can be done by the use of *in vitro* and *in vivo* evolutionary approaches.

2. Creation of synthetic biological circuits – using various macromolecules (enzymes, gene expression regulators, etc.) combined in a certain way, regulatory circuits with specific properties, such as oscillators, logic switches, etc. can be created in cells. That in turn allows cells to react in new ways to various stimuli. The creation of such regulatory circuits is enabled by molecular design, information technology and the principles of electronic engineering.

3. Creation of new metabolic pathways – new chemical processes can be introduced in the cell where new substances can be synthesized or metabolized, e.g. pollutants with a long life into the environment. The creation of those new metabolic pathways is enabled by the principles of organic chemistry coupled with design of macromolecules with new properties and new regulatory chains.

4. Extension of the repertoire of building blocks of biological macromolecules, beyond those naturally occurring amino acids and nucleotides – the combination of the above three areas should allow the metabolic synthesis of new amino acid and nucleotides and their inclusion into cellular proteins and nucleic acids and hence the extension of their possible functions.

5. Synthetic genomics – it is currently possible to syntheses *in vitro* complete viral and prokaryotic genomes and eukaryotic chromosomes that in some cases can be successfully introduced into the cell to substitute or supplement its natural genetic material. That allows the genome as a whole to have precisely defined, pre-determined sequence, and hence specific properties.

6. Minimal and synthetic cells and viruses – those studies aim to define the minimum requirements for the existence of cell-like or virus-like life forms using knowledge from the field of physical chemistry, biochemistry, cellular biology and evolutionary biology.

7. Ecosystem engineering – it is possible in principle to introduce into the environment organisms with such modifications that allow interactions within their population or with populations of other species to be changed in a specific ways. Gene drives are an example for such an approach.

The key enabling technologies in the context of Synthetic biology include *inter alia*:

1. techniques for DNA sequencing, sequence assembly and annotation that allow very large amounts of data to be generated and interpreted in short time and at low price;

2. techniques for DNA synthesis that allow large fragments of DNA to be produced *in vitro* accurately and cheaply;

3. techniques for *in vivo* manipulation of genomic DNA that allow *inter alia* simultaneous introduction of small changes at multiple well-defined sites;

4. techniques for *de novo* design of biological macromolecules, metabolic pathways or other biological process with predetermined properties.

Specific comments on the requests for information in Notification 2018-103

a) The relationship between synthetic biology and the criteria set out in decision IX/29, paragraph 12

Most of the technologies that can be considered to fall within the definition of Synthetic biology will not fulfil the criteria for new and emerging issues set in paragraph 12 of Decision IX/29. Gene drives are understood to be "systems of biased inheritance in which the ability of a genetic element to pass from a parent to its offspring through sexual reproduction is enhanced" (Gene Drives on the Horizon, 2016, ISBN 978-0-309-43787-5). Whether or not the gene drives fall with the scope of Synthetic biology is somewhat controversial, but that is not actually relevant for the question whether they can be considered a new and emerging issue with the context of the Convention or not. It should be noted that concept behind the gene drives is not new but recent technological developments (Reviewed in Cheating evolution: engineering gene drives to manipulate the fate of wild populations, Champer, Buchman and Akbari, Nature Reviews Genetics 17, 146-159) raise the practical possibility to eliminate or alter genetic structure of whole natural populations. Bellow we examine the gene drives against each of the criteria for a new and emerging issue assuming that the gene drives actually work as claimed and expected:

(a) Relevance of the issue to the implementation of the objectives of the Convention and its existing programmes of work;

Gene drives can, at least in theory, remove whole populations from the ecosystems or can significantly alter their genetic structure. Thus they can affect the conservation of biological

diversity directly (by removing or altering whole species or populations) or indirectly (e.g. by changing the relations between species), both negatively (e.g. directly removing whole populations or indirectly by removing the food base of some non-target species) or positively (e.g. by elimination invasive alien species, vectors or pathogens). The objective of the sustainable use of biological diversity components can also be affected positively (e.g. by eliminating important pests) or negatively (e.g. by affecting species used by local communities).

Taking into account the above considerations the criterion can be considered fulfilled.

(b) New evidence of unexpected and significant impacts on biodiversity;

The practical experience with release of gene drives into the environment is very limited. At the same time the modelling studies show that even very inefficient gene drives are likely to be highly invasive and practically impossible to control (Current CRISPR gene drive systems are likely to be highly invasive in wild populations, Noble et al., eLife 7, e33423). Taking into account that the relations between species in an ecosystem are non-linear and complex, elimination or altering of genetic structure of a whole natural population may result in effects whose nature or magnitude will be very hard to predict.

Taking into account the above considerations the criterion can be considered fulfilled.

(c) Urgency of addressing the issue/imminence of the risk caused by the issue to the effective implementation of the Convention as well as the magnitude of actual and potential impact on biodiversity;

As mentioned above the concept behind the gene drives is not new, but it seems likely that in near future organisms that carry efficient gene drives might be released into the environment. Currently no effective technology to control gene drives is available. The magnitude of actual or potential impacts on the biodiversity is hard to predict but is might to be significant (see point b) above) at least in some cases.

Taking into account the above considerations the criterion can be considered fulfilled.

(d) Actual geographic coverage and potential spread, including rate of spread, of the identified issue relating to the conservation and sustainable use of biodiversity;

The actual geographic coverage and potential spread, including rate of spread are likely to be depend of different factors, such as the genetic structure of the populations (e.g. the presence and frequency of resistance alleles), effective size and geographic spread of populations affected, reproduction times, etc. In some cases, such as mice or mosquitoes, the potential effects can be global.

Taking into account the above considerations the criterion can be considered fulfilled.

(e) Evidence of the absence or limited availability of tools to limit or mitigate the negative impacts of the identified issue on the conservation and sustainable use of biodiversity;

Different approaches to control or eliminate gene drives that have been released into the environment have been proposed (For some examples see again Champer, Buchman and Akbari, Nature Reviews Genetics 17, 146-159) but so far we are not whether any of them to have proven to be effective in natural context. So for the time being the released of gene drives into the environment should be considered irreversible (taking into account the stochastic nature of the process of establishment, which is likely to be efficient or the self-elimination due to the eradication of the whole target population).

Taking into account the above considerations the criterion can be considered fulfilled.

(f) Magnitude of actual and potential impact of the identified issue on human well-being;

No gene drives have been released into environment in a large scale, so only potential impact can be considered at present. Those impacts can be both positive (e.g. elimination of pathogens, pests or vectors for important diseases, such as malaria) or negative (e.g. effects of some pollinators) and of significant magnitude.

It should be noted that formally this criterion allows the actual and potential impact to be either positive or negative. Thus it can be considered fulfilled.

(g) Magnitude of actual and potential impact of the identified issue on productive sectors and economic well-being as related to the conservation and sustainable use of biodiversity;

Similar to the issue above, only potential impact can be considered. Those impacts can be either positive (e.g. increased crop yields due to pathogens or pests elimination) or negative (e.g. effects of some pollinators) and of significant magnitude.

It should be noted that formally this criterion allows the actual and potential impact to be either positive or negative. Thus it can be considered fulfilled.

Our overall conclusion is that the gene drives are very likely to fulfil formally all the criteria for a new and emerging issue as set out in decision IX/29, paragraph 12.

b) New technological developments in synthetic biology since the last meeting of the Ad Hoc Technical Expert Group, in order to support a broad and regular horizon scanning process

Our analysis covers the published literature in the period December 2017 – January 2019 and is by no means complete and only some more significant articles in the field of Synthetic biology are highlighted:

1. Circuit design features of a stable two-cell system, Zhou *et al.*, Cell 172 (4), 744-757
This paper demonstrates the principles on which stable cell-circuits can be formed. Similar principles can be used to design novel cell-circuits that do not exist naturally.

2. Evolutionary Convergence of Pathway-Specific Enzyme Expression Stoichiometry, Lalane *et al.*, Cell 173 (3), 749-761

This paper identifies an important principle for building biological pathways that can significantly facilitate development of new synthetic pathways.

3. High-Throughput Investigation of Diverse Junction Elements in RNA Tertiary Folding, Knight Denny *et al.*, Cell 174 (2), 377-390
This paper demonstrates the relationships between sequence, structure and energetic in RNA and can provide basis improved design of RNA molecules with specific functions, e.g. switches.

4. A Synthetic Bacterial Cell-Cell Adhesion Toolbox for Programming Multicellular Morphologies and Patterns, Glass and Riedel-Kruse, Cell 174 (3), 649-658
Genetically encoded synthetic platform for modular cell-cell adhesion in Escherichia coli is reported, which provides control over multicellular self-assembly and allows for quantitative rational design of well-defined morphologies and patterns.

5. Scalable, Continuous Evolution of Genes at Mutation Rates above Genomic Error Thresholds, Ravikumar *et al.*, Cell 175 (7), 1946-1957
A system for scalable, continuous evolution of user-defined genes in vivo is described that allows routine, high-throughput evolution of biomolecular and cellular function to be carried out.

6. Engineering Epigenetic Regulation Using Synthetic Read-Write Modules, Park *et al.*, Cell 176 (1), 227-238
A synthetic epigenetic regulatory system in human cells using m6A DNA modification that allows construction of regulatory circuits is described.

7. Small-Molecule Agonists of *Ae. aegypti* Neuropeptide Y Receptor Block Mosquito Biting, Duvall *et al.*, Cell 176 (4), 687-701
Although not utilizing synthetic biology, the paper demonstrates how small-molecule compounds can be used to control disease vectors through altering their behavior.

8. Biotechnological mass production of DNA origami, Praetorius *et al.*, Nature 552 (7683) 84-87
This paper presents a method for scalable productions of large DNA molecules of arbitrary sequence that can be used for production of complex nanostructures.

9. Genetically programmed chiral organoborane synthesis, Kan *et al.*, Nature 552 (7683) 132-136
A genetically encoded platform for producing chiral organoboranes in bacteria that expands the chemical reactions that can be carried out in cell is presented.

10. Evolution of a designed protein assembly encapsulating its own RNA genome, Butterfield *et al.*, Nature 552 (7685), 415-420
The paper presents the development of synthetic nucleocapsids that can package their own RNA genome. This is an example of *de novo* design of virus-like structures.

11. Optogenetic regulation of engineered cellular metabolism for microbial chemical production, Zhao *et al.*, Nature 555 (7698), 683-687
The paper demonstrates the use of engineered metabolic pathways to control fermentation with only light.

12. Creating a functional single-chromosome yeast, Shao *et al.*, Nature 560 (7718), 331-335
Creation of single chromosome yeast is presented. This demonstrates the feasibility of chromosomal engineering.

13. Karyotype engineering by chromosome fusion leads to reproductive isolation in yeast, Luo *et al.*, Nature 560 (7718), 392-396
Work similar to the one above; another demonstration of practicability of chromosomal engineering.

14. In vivo CRISPR editing with no detectable genome-wide off-target mutations, Akcakaya *et al.*, Nature 561 (7723), 416-419
A highly sensitive strategy that can robustly identify the genome-wide off-target effects of CRISPR–Cas nucleases *in vivo* is described. The paper shows show that appropriately designed guide RNAs can direct efficient *in vivo* editing with no detectable off-target mutations.

15. De novo design of a fluorescence-activating β-barrel, Dou *et al.*, Nature 561 (7724), 485-491
The first demonstration of accurate *de novo* design of β-barrel proteins that can bind small molecule ligands and activate them.

16. Predictable and precise template-free CRISPR editing of pathogenic variants, Shen *et al.*, Nature 563 (7733), 646-651
The paper presents an approach for precise, template-free genome editing.

17. Controlling orthogonal ribosome subunit interactions enables evolution of new function, Schmied *et al.*, Nature 564 (7736), 444-651
An ingenious *in vivo* system that can be used to evolve proteins with new functions, incl. containing non-natural amino acids is described.

18. De novo design of potent and selective mimics of IL-2 and IL-15, Silva *et al.*, Nature 565 (7738), 186-191
The paper demonstrates that *de novo* design can used to develop potent and selective mimics of natural signaling molecules. Such molecules can be completely unrelated to the natural counterpart and might have superior therapeutic properties.

19. Super-Mendelian inheritance mediated by CRISPR–Cas9 in the female mouse germline, Grunwald *et al.*, Nature 566 (7742), 105-109
A gene drive system in mammalians is described.

20. Rewritable multi-event analog recording in bacterial and mammalian cells, Tang and Liu, Science 360 (6385), eaap8992
The paper describes a system that allows recording of multiple cellular events in the genome.

21. Cellular checkpoint control using programmable sequential logic, Andrews, Nielsen and Voigt, Science 361 (6408), eaap8987
The paper presents genetic circuits that encode sequential logic to instruct cells to proceed through a linear or cyclical sequence of states.

22. Programmable protein circuits in living cells, Gao *et al.*, Science 361 (6408), 1252-1258
The paper presents a scalable platform to facilitate protein circuit engineering for biotechnological applications based on orthogonal modular proteases.

23. Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field, South *et al.*, Science 363 (6422), eaat9077
The study demonstrates that engineered metabolic pathways can affect and improve the agricultural properties of plants.

In general, we think that such analysis of published literature performed by the Parties and/or can be only one element of a broad and regular horizon scanning process of the new developments in the field of modern biotechnology. We suggest that it is supplemented by regular (e.g. annual) request for information on the recent technological developments that affect the objectives of the Convention to be sent by the Secretariat to the leading academic departments and scientific institutions that are active in the relevant fields. This can be a very effective way to obtain up-to-date information from leading scientists.

c) The current state of knowledge on the potential positive and negative environmental impacts of current and near-future applications of synthetic biology

Some of the current applications of synthetic biology that may have environmental impacts have been mentioned in item b) above.

d) Living organisms developed thus far through new developments in synthetic biology that may fall outside the definition of living modified organisms as per the Cartagena Protocol

The relevant definitions of the terms are given in points g), h) and i) of Article 3 of the Cartagena Protocol.

A recent study demonstrates the creation of virus like entities consisting of computationally designed icosahedral protein assemblies capable of packaging their own full-length mRNA genomes (Evolution of a designed protein assembly encapsulating its own RNA genome, Butterfield *et al.*, Nature 552 (7685), 415-420). The created macromolecular assemblies are certainly obtained through the use of modern biotechnology. Those assemblies do not have all functions required for a complete viral life cycle and are not capable of transferring or replicating their genetic material. Thus they cannot be considered living organism, but it can be imagined that similar non-biological synthetic assemblies might be developed in the near future that will be capable to enter and replicate inside cells and be even more similar to naturally occurring viruses.