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Comment on EFSA' s draft on Synthetic Biology developments in micro-organisms, environmental risk assessment aspects (ERA)

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General comments

Applications of synthetic biology to micro-organisms (SynBio-MO), including, for example, bacteria, viruses, fungi and yeast, may become a threat to the global biosphere to greater extent compared to many other applications of genetic engineering of more complex species. Micro-organisms (MO) are the basis of life on earth and the common network of all existing species. They are essential for mobilising and exchanging abiotic and biotic resources. Further, they transmit biologically active molecules and information across boundaries between species. The emergence of diseases further adds to the complex interactions between MO and other species. MO reproduce fast compared to more highly developed species and are thus adept at spreading rapidly in their environments. Therefore, in dealing with SynBio MO, the precautionary principle deserves to be given the highest priority.

As EFSA correctly states: *"Even with the complete genetic information of a synthetic micro-organism, it is beyond the capacity of any existent bioinformatic analysis to fully predict the capability of a synthetic organism to survive, colonise and interact with other organisms under natural conditions, given the uncountable diversity of potential microhabitats and their temporal variability."* However, in the documents published for consultation, the role of the precautionary principle is neither mentioned nor explained and therefore not given the emphasis that should have been expected. This is one of the major deficiencies of the draft document (EFSA, 2020) as is the supporting report (van der Vlugt, 2020).

Overview of applications

There are an increasing number of projects which may include releases of SynBio MO into the environment. Some examples:

- Potential uses SynBio MO imply the engineering of ecosystems, microbial communities for purposes such as changing biodegradation, waste treatment and bioremediation (Wang et al., 2013; Mee et. al., 2014; Qian et al., 2020).
- Several projects aim to change gut microbiota in animals and humans (Mimee et al., 2015; Kim et al., 2018; Ronda et al., 2019). Some of these approaches are being discussed in therapeutic concepts (Mimee et al., 2016; Sheth et al., 2016; Bober et al., 2018; Ozdemir, 2018; Hwang & Chang, 2020).
- Other applications directed at food and feed aim to change the composition of diets and products for human consumption (Lee et al., 2016; Mertens et al, 2019).
- SynBio or GE applications to change gut microbiota are also being discussed for insects such as flies (De Vooght et al., 2014; Gilbert et al., 2016) mosquitoes (Ren et al., 2008; Fang et al., 2011; Bilgo et al., 2017; Lovett et al., 2019) and bees (Rangberg et al., 2012; Leonard et al., 2018; Leonard et al., 2020). Some of these approaches are called 'paratransgenesis', which means the biological characteristics of a target host are changed by genetically

engineering its symbiotic bacteria, for example, to eliminate a pathogen from insects via the expression of effector molecules (Wilke et al., 2015).

- Similar approaches are being discussed in regard to corals (Levin et al., 2017).
- In agriculture, there are ongoing discussions in regard to applications that change the microbiomes of plants, e.g. mycorrhiza or endophytes (Vorholt et al., 2017; Checcucci et al. 2018; Hettiarachchige et al., 2019; Arif et al., 2020).
- In agriculture, SynBio applications for soil microorganisms are also being discussion (Temme et al., 2012; Shelake et al., 2019; Shulse et al., 2019).
- Further potential uses include the usage of SynBio MO as pesticides (Leclère et al., 2005; Tseng et al., 2005; Wang et al., 2011; Fang et al., 2014; Scheepmaker et al., 2016; Azizoglu et al., 2020).
- Several projects are looking at usages of SynBio MO (such as cyanobacteria or algae) for energy production (Wang et al., 2013; Nozzi et al., 2013; Motomura et al., 2018).
- Other applications include viral systems, such as bacteriophages (Citorik et al., 2014; Lemire et al., 2018), and even the dissemination of genetically engineered viruses via insects ('insect allies') for potential military purposes (Reeves et al., 2018).

Methods available to generate SynBio MO include tools such as (i) synthesis of the genome in part or as a whole (*de novo* synthesis), (ii) the combination of genetic elements from natural or artificial sources, (iii) deletions or changes in the natural genome, (iv) the introduction of artificial genetic elements like new 'letters' of DNA (xeno-nucleic acid, XNA) and (v) the construction of artificial chassis. Genome editing and DNA synthesis techniques are increasingly being used in synthetic biology and are a major driver in the development of new SynBio MOs. Beyond such applications in the laboratory, it has to be considered that biological characteristics of MO may also become engineered in wild populations, e.g. by application of short nucleotides such as dsRNA (for some overview see: Christiaens et al., 2020).

However, the documents published by EFSA only discuss a narrow range of selected examples, in total just 45 cases. The horizon scanning performed by van der Vlugt (2020) mentions more than 700 relevant publications, but ends up discussing just a selection of 11 examples with some of these simply referenced. A more in-depth investigation of the presented and additional cases is required to cover the complexity of SynBio MOs. Furthermore, EFSA should aim to give a much more comprehensive overview of all applications involving potential releases of SynBio MO and related techniques, also including medical purposes if these are connected to releases into the environment. Upon release, SynBio MOs can survive and persist in the receiving environment or invade new environments where they can have multiple interactions with other organisms; all this needs in-depth investigation.

Missing: the concept of the holobiont

There is a general methodological problem in the approach of EFSA: many microorganisms are closely associated with other species and often considered to be 'hosts'. For example, MO are part of the microbiome of plants, insects, mammals, humans. Under such circumstances, the biological effects, and potential adverse effects of SynBio MO will emerge from these symbiotic interactions in a non-linear pattern. Therefore, these biological systems cannot be assessed just by looking at single parts and pieces in isolation, they all have to be considered as larger units known as holobionts or hologenoms, taking into account that all species in the same habitat interact and influence each other (see for example Richardson, 2017; Sanchez-Canizares, 2017; Arif et al., 2020). Because EFSA does not consider this concept, its consideration of possible interactions remains fragmentary: it is not only the SynBio microorganisms which may act upon target and non-target organisms, but also the host and the hologenome may impact the characteristics of the SynBio microorganism. Furthermore, risk assessment of genetically engineered hosts, which may be combined with a SynBio microorganism by accident or on purpose, also need to be considered.

As yet the concept of holobionts (or hologenomes) is not taken into account. This is a general and major flaw in all approaches to GMO risk assessment carried out by EFSA. Without integrating this level of complexity, risk assessment of genetically engineered organisms, including market applications for transgenic plants for consumption or cultivation, remains inconclusive (see Bauer-Panskus et al., 2020a).

Whatever the case, EFSA should extend its considerations regarding what they call the “systems approach”, that should be performed “from a holistic point of view” (EFSA, 2020), to include the concept of the hologenomes and holobionts. This could fundamentally strengthen their recommendations. Further, within these concepts, EFSA should not only consider horizontal gene transfer, but also other exchanges of biologically active molecules, such as dsRNA, which are transmitted beyond biological kingdoms, impacting gene regulation of both the associated MO and its host.

Missing: more detailed scenarios for risk assessment

EFSA considers five case studies of SynBio MOs to evaluate the adequacy of already existing guidelines for risk assessment and whether updated guidance is needed. The five examples are analysed only partially, without going into details. Without exploring much more detailed scenarios, EFSA’s draft document may give the impression that risks might be assessed by modelling, whole genome sequencing, experiments in the laboratory and any small-scale releases. A more in-depth analysis of the case studies and further scenarios needs to be carried out to ensure robust risk assessment.

For example, there are plans to use genome editing on the associated symbiotic microorganisms of corals in order to strengthen their ability to adapt to climate change and higher temperatures (Levin, 2017). Corals are complex organisms, holobionts that are dependent on a symbiotic relationship with algae and other microorganisms, e.g. bacteria and archaebacteria, that produce substances essential for their life and survival (Rosenberg & Rosenberg-Ziller, 2016). It is believed that in coral bleaching due to climate change, disruption of the symbiosis between the multicellular body of the corals (the polyps) and their endosymbiotic microalgae (*Symbiodiniaceae spp*) plays an important role. Initial studies are already developing ideas on how new genetic engineering methods can be applied to protect microalgae (*Symbiodiniaceae spp*) from the effects of heat-induced damage (Levin et al., 2017). Rosenberg & Zilber-Rosenberg (2016) describe how the symbioses contribute to genetic diversity of corals as well as their evolutionary adaptation. They refer to research showing that the composition of the associated microorganisms can change spontaneously in altered environmental conditions, and therefore enable the adaptation of the corals in water that is warmer by one or two degrees (see, for example, Oliver & Palumbi, 2011). Other research has reported a surprising natural adaptability of corals (Kersting & Linares, 2019; Kenkel & Matz, 2016). The various mechanisms with which specific coral systems can adapt to climate change are still not clearly understood. For example, it is not predictable how interactions between the corals and their symbionts could be influenced through genetic engineering. As a result, there is an extremely high degree of uncertainty and non-knowledge, which is likely to make any risk assessment inconclusive. In addition, there is the problem that the genetically engineered holobionts cannot be removed from the coral colonies after release. Therefore, it will not be possible to intervene if harmful long-term consequences occur; this is in contradiction to the precautionary principle (see also Testbiotech, 2020).

Other cases with similar complexity include plans to genetically engineer gut bacteria in honeybees, to produce biologically active molecules (dsRNA) to render toxic effects beyond the biological kingdoms (Leonard et al., 2020). The dsRNA was designed to target varroa mites and the deformed wing virus to protect honeybees from infection. The genetically altered bacteria are transmitted via direct contact with honeybees. Thus, it is, for example, important to comprehensively investigate

whether the genetically engineered bacteria can also be transmitted to other organisms. Furthermore, to exclude the possibility of unwanted spread, there needs to be investigation into whether the genetically altered bacteria are capable of surviving outside of the gut.

Another example that is mentioned in the associated horizon scan of the EFSA opinion (van der Vlugt, 2020) but not sufficiently analysed, is a study that reports the release of fungal organisms producing spider toxins to kill mosquitoes (Lovett et al., 2019). Obviously, other insects can also be infected with the transgenic fungus, but empirical data on possible spread and its impact on non-target organisms is still missing in that regard. EFSA should expand the draft document to include such scenarios and explore risks, uncertainties and non-knowledge in much more detail. Otherwise, there is a danger of creating the impression that such interventions into complex systems could be established in the near future and sufficiently risk assessed before releases take place.

Inclusion of cut-off criteria in the risk assessment

In this context, EFSA should also address spatio-temporal control and traceability. It is known that GE organisms can react to environmental stressors in unexpected ways and may show next generation effects which cannot be predicted from the original generations produced in the lab (see Bauer-Panskus et al., 2020b). In the context of SynBio MO, risk assessment of any next generation effects has to take the concept of holobionts and hologenomes and their reactions to the environment over longer period of time into account.

Also EFSA, in its draft document, states that in many cases, available information and risk assessment methodology will not be able to "*fully predict the capability of a synthetic organism to survive, colonise and interact with other organisms under natural conditions, given the uncountable diversity of potential microhabitats and their temporal variability.*" Against this background, EFSA should introduce a set of cut-off criteria which will allow decisions to be made on the conclusiveness of risk assessment (see Then et al., 2020). If risk assessment is identified as inconclusive, no release can be permitted because it would not be compatible with the precautionary principle.

Traceability has to become part of the safety concept

In the light of the Corona pandemic and with a heightened sense of urgency, EFSA should also consider elements which can be used to track and trace SynBio MO organisms that might escape unnoticed from the laboratory. EFSA claims it will use a unique barcoding sequence for any SynBio MO to properly identify and detect them if released. Additionally, international databases are needed which can be used to identify all organisms (applicable to SynBio MOs, GE plants, GE animals) generated or modified by means of gene synthesis, genome editing or transgenesis and which might be released or escape into the environment. These databases are indispensable to investigate putative interactions between multiple genetically engineered organisms. The fate of such organisms in the environment and their genetic material should be made traceable for experts and authorities all around the world. In addition, whole genome sequencing (WGS) data should be made available for international registration.

Further, for micro-organisms which may cause diseases in plants, animals or humans (or other group of organisms) these databases should also incorporate and store specific information data on gene synthesis performed in the lab. If the genome of a pathogen is constructed, synthesized and assembled using gene synthesis methods in a lab, the information on such experiments needs to be unrestrictedly stored in a control system to prevent potential dual-use of these DNA sequences (see EGE, 2009; Testbiotech, 2010). Additionally, such data will not only allow the potential origin of new viruses to be determined, but also avoid a situation as mentioned by van der Vlugt (2020): according to this survey, SynBio organisms might have already been released in the US for agricultural purposes, without any information about their molecular and biological characteristics.

In the EFSA opinion, genetic firewalls are discussed as control measures to achieve containment by auxotrophy, and to ensure that SynBio MOs are not able to independently spread in the environment without the application of natural or synthetic substances to survive (e.g. the dependence on novel amino acids or xeno-nucleic acids). But a major concern, which was not addressed in the EFSA opinion, was that after reproducing over several generations such micro-organisms can develop a mutation that allows them to survive unrestricted without these substances.

Conclusions

EFSA should emphasize that protection of human health and biodiversity as well as the precautionary principle have to become a priority before SynBio MO are considered for release. In this context, cut-off criteria for risk assessment have to be developed, which allow decision-making in the light of non-knowledge and uncertainties.

Further, traceability has to be developed as part of safety regulations, new standards of storage and transparency of data are urgently required, and must also be considered in the context of risk assessment.

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