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To the Minister of
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REFERENCE CGM/190509-01
SUBJECT Letter of advice 'Containment level for laboratory experiments with gene drives' and presentation of research report

Dear Mrs Van Nieuwenhuizen,

In 2015 several publications appeared on genetically modified organisms that had been altered in such a way that the modification would be able to spread rapidly throughout a population. These organisms were modified using a 'gene drive' based on the CRISPR-Cas9 system. If an organism carrying such a gene drive were to escape from the laboratory into a natural population, it was claimed, this would lead to an irreversible change in the genetic makeup or eradication of the whole population or species. For this reason, in the Netherlands laboratory experiments with CRISPR gene drives were assigned to the highest containment level.[1,2] However, there are doubts about the ability of gene drives to efficiently propagate themselves throughout natural populations and therefore whether this high containment level is justified.[3]

Gene drives also occur in nature in various organisms, such as fruit flies, mosquitoes and mice, and these gene drives have been the subject of research for some decades. As insights gained from this research are relevant to assessing the risks of 'synthetic' gene drives, COGEM carried out a literature study of these earlier experiments. At the same time, the available knowledge about 'synthetic' gene drives was reviewed.

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[1] Staatscourant (2016). Regeling van de Staatssecretaris van Infrastructuur en Milieu van 15 juni 2016, nr. IENM/BSK-2016/107788, tot wijziging van de Regeling genetisch gemodificeerde organismen milieubeheer 2013 (aanpassing bijlagen 2,4, 5 en 11). Nr. 31027; 16 juni 2016
[2] Kamerbrief (2016). Beleid ten behoeve van een nieuwe ontwikkeling in de biotechnologie. Kenmerk IENM/BSK-2015/225461
[3] DeFrancesco L (2015). Gene drive overdrive. Nat. Biotechnol. 33(10): 1019-1021

This study revealed that a small number of field experiments have been carried out with gene drives used to control insects (almost always mosquitoes), with varying degrees of success. In none of the field experiments were adverse effects on humans or the environment detected and the gene drives did not spread to other populations. Various factors can hinder the spread of gene drives, such as the biology and population dynamics of the organism concerned, the efficiency of the gene drive itself and the 'fitness costs' associated with the gene drive. No field experiments have yet been carried out on 'synthetic' gene drives. The available data indicate that insects can easily become resistant to CRISPR gene drives, thus impeding their propagation throughout the population.

Based on this literature study, and information on the development of resistance to gene drives in particular, COGEM concludes that should a single organism carrying a CRISPR gene drive escape from a laboratory, the probability of a whole population being genetically modified or eradicated as a consequence would be negligible. COGEM advises limiting the types of experiments with CRISPR gene drives that are allocated to the highest containment level category to those organisms with CRISPR gene drives that have been specifically designed to prevent the emergence of resistance or to overcome resistance.

Background

In 2016 it was decided to amend the Ministerial Regulation on Genetically Modified Organisms (GMO Regulation) 2013 to require activities that may lead to the production of an organism carrying a CRISPR gene drive system to be allocated to the highest containment level (category IV). The reason for this decision was that the escape from a laboratory of an organism carrying a CRISPR gene drive would lead to the irreversible propagation of the CRISPR gene drive throughout a whole population, with possible serious environmental consequences.[1,2] However, at that time a number of scientists already had doubts about the ability of gene drives to efficiently propagate themselves throughout natural populations.[3]

Gene drives occur naturally in various organisms and research into how they can be used to control unwanted insects, such as malaria mosquitoes, has been ongoing for decades. To obtain insight into what has been learned from these studies, including about the propagation of gene drives, COGEM commissioned a literature study. The study was carried out by Perseus BVBA and resulted in the accompanying research report 'Gene Drives – Experience with gene drive systems that may inform an environmental risk assessment' (CGM 2018-03).

Research results

Research into gene drive systems

Gene drives occur in nature in various organisms, including fungi, plants, insects and mammals. Different gene drive systems have been found in these organisms, such as transposons, underdominance, MEDEA, homing endonucleases and meiotic drives. In normal sexual reproduction, a specific genetic sequence is passed on to half of the offspring. Gene drives, however, are passed on to more than half of the offspring, increasing the frequency of the gene drive in the population. The degree to which the frequency is increased depends on the gene drive itself, the organism and environmental factors.

Research into the use of gene drives to alter the genetic makeup of organisms in a population (called 'modification drives' or 'replacement drives') or control the organisms in a population (called 'suppression drives') has been ongoing since the 1970s. Most gene drive systems have only been studied in the laboratory. A few gene drive systems – underdominance gene drives (cytoplasmic incompatibility and translocations) and the symbiotic bacterium *Wolbachia*[4] – have been field tested as a mechanism for controlling pest insects (mostly mosquitoes). In these field experiments the target population was not entirely eradicated or replaced. The reasons for this include low fitness of the organisms carrying the gene drive, releasing too few organisms carrying the gene drive, and immigration of insects from neighbouring populations. In these field experiments the gene drives did not spread to other populations.

Synthetic gene drives

The research report also discusses synthetic gene drives, such as CRISPR gene drives. CRISPR gene drives are a form of homing endonuclease gene drives and can be used both to alter the genetic composition of organisms in a population (modification drives) and to control or suppress organisms in a population (suppression drives). In some cases, the CRISPR gene drive itself directly induces the desired effect; in other cases, an effector gene that induces the effect (called the 'cargo' or 'payload gene') is linked to the CRISPR gene drive.

CRISPR gene drives are highly efficient under laboratory conditions, but have not yet been tested in the field. The researchers observe that the highly specific mode of action of CRISPR gene drives can easily lead to the emergence of resistance and the report contains various examples of laboratory experiments in which organisms developed resistance to the gene drives. The researchers also note that natural populations may contain organisms with a natural resistance to CRISPR gene drives.

[4] Although the bacterium *Wolbachia* strictly speaking does not fully meet the definition of a gene drive, it propagates itself in the same manner and for this reason was included in the research.

Dispersal of gene drives in the environment

In the earlier field experiments the gene drives did not spread to other populations. The researchers mention various factors that can impede the propagation of gene drives, such as the biology and population dynamics of the organism concerned, the efficiency of the gene drive and the fitness costs associated with the gene drive and the cargo gene (if present). The spread of a gene drive can also be impeded by the presence of resistant organisms, which may be naturally present in the population or emerge due to the mode of action of the gene drive. The researchers therefore consider it unlikely that the introduction of an organism carrying a gene drive will inevitably lead to the eradication or genetic modification of all organisms of a particular species.

Recent research into reducing the development of resistance

CRISPR gene drives make an enzyme, endonuclease, which cuts the DNA at a specific site so that the gene drive can be transferred. When the cell repairs this break a sequence may be created that is not recognised by the gene drive, which cannot then be transferred to the organism or passed on to its offspring; the organism has in effect developed resistance to the gene drive. Because resistant organisms hinder the spread of gene drives, researchers are investigating how the development of resistance can be reduced or prevented.

To minimise the emergence of resistance, research is being done that aims to create gene drives in which the presence of endonuclease is limited to the phase in which the gene drive is transferred (during the formation of gametes).[5] However, the above is not sufficient to entirely prevent alteration of the recognition sequence, which is why gene drives are being developed that recognise multiple sequences in the DNA.[5,6] A change in one of the recognition sequences will then not immediately confer resistance, because the gene drive can still be transferred via the other recognition sequences. Although such gene drives do reduce the emergence of resistance, they do not entirely prevent the emergence of resistance.[5,6] One reason is that these gene drives sometimes make use of just one of the recognition sequences. These gene drives also appear to be less efficient and are often only partly transferred.[5,6] Apparently, it is particularly difficult to develop a gene drive that does not induce any resistance at all and no such gene drives have yet been created.

Special gene drives are also being developed that make it impossible for resistant organisms to reproduce, thus preventing the emergence of resistance from impeding the propagation of the gene drive. In two experiments with a gene drive designed to make resistant mosquitoes sterile, both gene drives were able to eradicate a group of malaria mosquitoes (*Anopheles*

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[5] Champer J *et al.* (2018). Reducing resistance allele formation in CRISPR gene drive. PNAS 115 (21): 5522-5527

[6] Oberhofer G *et al.* (2018). Behavior of homing endonuclease gene drives targeting genes required for viability or female fertility with multiplexed guide RNAs. PNAS 115 (40): E9343-E9352

gambiae) held in a net cage.[7] This result was achieved within a limited number of generations (8 and 12) because a relatively large number of mosquitoes with the gene drive were present at the start of the experiment and the total number of mosquitoes was relatively small. Although in some of the mosquitoes the gene drive recognition sequence was altered, these mosquitoes were unable to reproduce and the rate of propagation of the gene drive was unaffected. The researchers note that further research is needed to determine whether or not this gene drive is also able to eradicate a wild population of mosquitoes.

Research with GM mosquitoes shows that under natural conditions these mosquitoes are often less fit than wild-type individuals of the same species, which could hinder the propagation of the gene drive. Moreover, the genetic diversity is much greater in mosquitoes from natural populations, which means that these populations can contain mosquitoes that do not have the sequence recognised by the gene drive and are therefore resistant to the gene drive. The researchers have tried to overcome this by using a recognition sequence for their gene drive which is present in almost all malaria mosquitoes (*A. gambiae* complex). However, some wild malaria mosquitoes (2.9% of 756 captured malaria mosquitoes from Sub-Saharan Africa) have been found to carry a version of this recognition sequence that is not exactly the same, but which differs by a single nucleotide. This sequence is still recognised by the gene drive,[7] but it cannot be ruled out that elsewhere malaria mosquitoes exist that have a sequence that is not recognised by the gene drive. The researchers observe that in time resistance to their own gene drive could also emerge. It is not impossible that a mosquito will eventually emerge with the recognition sequence altered in such a way that it cannot be recognised by the gene drive, but which still remains fertile.[7]

COGEM advice

On the basis of the research results, COGEM concludes that the notion that once gene drives have entered the environment they will inevitably eventually eradicate or modify species or populations is, in reality, not as straightforward and requires qualification. The number of field trials that have been carried out on gene drives is limited, but there are no indications that gene drives can propagate unhindered throughout populations in the wild.

CRISPR gene drives have not yet been investigated in the field, but resistance to these gene drives emerges easily. At the moment, research is being conducted into CRISPR gene drives that can reduce or overcome resistance, but it has not yet proved possible to develop a well-functioning CRISPR gene drive that has not eventually induced the development of resistance among the target population. However, a CRISPR gene drive has been made that prevents organisms that do develop resistance from being able to reproduce, so that propagation of the gene drive through the population is not hindered. The potential of this gene drive to propagate throughout wild mosquito populations under natural circumstances has not yet been investigated.

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[7] Kyrou K *et al.* (2018). A CRISPR-Cas9 gene drive targeting doublesex causes complete population suppression in caged *Anopheles gambiae* mosquitoes. Nat. Biotechnol. 36: 1062-1066

Based on the literature study, COGEM observes that concerns about researchers unintentionally creating a CRISPR gene drive that could pose environmental risks are unfounded. There are no indications that if an organism carrying a CRISPR gene drive escaped from a laboratory it could lead to the eradication or modification of a whole population. It is even questionable whether CRISPR gene drives that have been specially designed to prevent or overcome the emergence of resistance will be able to spread unimpeded throughout natural populations and have a major impact. However, for the time being it cannot be ruled out that these CRISPR gene drives could have such effects.

In view of the above, COGEM advises limiting the types of experiments with CRISPR gene drives that are allocated to the highest containment level category to those organisms with CRISPR gene drives that have been specifically designed to prevent the emergence of resistance or to overcome resistance.

Yours sincerely,

Professor Sybe Schaap
Chair of COGEM

c.c. Dr J. Westra, GMO Office
J.K.B.H. Kwisthout, Ministry of Infrastructure and Water Management