



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Gene drives

Policy report

RIVM Letter report 2016-0023
J. Westra et al.



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Colophon

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Gene drives

Gene drives zijn genetische eigenschappen die zodanig in het DNA van een organisme zijn ingebouwd dat ze aan alle nakomelingen worden doorgegeven, in plaats van aan een deel. Dit werkt ook door in de volgende generaties. Vooral als organismen zich snel voortplanten, kan deze eigenschap zich snel en blijvend in een hele populatie van een organisme verspreiden. Dit kan tot belangrijke innovaties leiden, maar gaat ook gepaard met zorg. Uit een analyse van het RIVM blijkt dat de huidige methoden voor het beoordelen van de risico's voor mens en milieu voor de effecten van gene drives onvoldoende geschikt zijn. Het RIVM adviseert daarom om alle toepassingen van gene drives in laboratoria expliciet onder de vergunningplicht van de ggo-wetgeving te brengen. Een melding volstaat niet.

Wettelijk gezien is een organisme met een gene drive een genetisch gemodificeerd organisme (ggo), waarvoor in Nederland een vergunnings- of meldingsplicht bestaat. Er mag alleen met ggo's worden gewerkt als de risicobeoordeling laat zien dat de risico's voor mens en milieu verwaarloosbaar klein zijn.

De huidige beoordelingsmethode is niet of ten dele toegesneden op ggo's met een gene drive, omdat onvoldoende rekening wordt gehouden met de snelle en blijvende verandering van de hele populatie. Ook kan in incidentele gevallen een gene drive per ongeluk ontstaan doordat onderzoekers genetische componenten onbedoeld zodanig toepassen dat een gene drive wordt gevormd.

Verder beveelt het RIVM aan de huidige regelgeving zo aan te passen dat het niet langer mogelijk is om onbedoeld een gene drive te maken. Verder kan een vergunning uitsluitend verleend worden als de benodigde gegevens beschikbaar zijn en daarmee alle vragen uit de risicobeoordeling kunnen worden beantwoord. Hiermee is een veilige toepassing van organismen met een gene drive geborgd en groeit kennis over de werking en de gevolgen van een gene drive. Ten slotte is een internationale aanpak gewenst omdat het kan gaan om organismen en mogelijke effecten op mens en milieu die zich over de landsgrenzen heen kunnen verspreiden.

Een voorbeeld van een mogelijke toepassing van een gene drive is een malariamug die door genetische aanpassing geen parasiet meer kan overdragen. Hierdoor kan deze eigenschap zich snel in de muggenpopulatie verspreiden en kan malaria eenvoudiger bestreden worden.

Kernwoorden: gene drive, genetisch gemodificeerde organismen, ggo, risicobeoordeling, ggo-wetgeving, milieu, DNA, populaties

Synopsis

Gene drives

A gene drive is a genetic trait that is built into the DNA of an organism in such a way that it is passed on to all its offspring, instead of only to some of the offspring. This trait is passed down through the generations. This trait can spread quickly and permanently through an entire population, especially when organisms reproduce rapidly. The use of gene drives can lead to important innovations but is also a cause for concern. An analysis by RIVM indicates that current methods for assessing the risks to human health and the environment are less suitable for the effects of gene drives. RIVM recommends that authorisation should be obligatory for applications in laboratories of organisms with a gene drive. Notification is insufficient.

In terms of the law, an organism with a gene drive is a genetically modified organism (GMO), for which an authorisation or notification is mandatory in the Netherlands. Working with GMOs is only permitted if a risk assessment shows that the risks to human health and the environment are negligible.

The current assessment method is not or partially tailored to GMOs with a gene drive, because the rapid and permanent alteration of the entire population is insufficiently taken into account. Sporadically, a gene drive may occur by accident when researchers apply genetic components in such a way that they inadvertently form a gene drive.

RIVM furthermore recommends adapting current legislation so that it is no longer possible to inadvertently create a gene drive. In addition, authorisation should only be granted if sufficient information is available to answer all questions in the risk assessment. This will ensure the safe use of organisms with a gene drive and provide an opportunity to gain knowledge about the way gene drives work and their impact. Finally, an international approach should be sought since this may concern organisms and potential effects on human health and the environment that could spread across national borders.

An example of a possible application of a gene drive is a genetically modified malaria mosquito that is no longer able to transmit the malaria parasite. Spreading this trait rapidly throughout the mosquito population using a gene drive would enable malaria to be controlled more easily.

Keywords: gene drive, genetically modified organisms, GMO, biosafety, risk assessment, GMO legislation, environment, DNA, populations

Contents

Summary — 9

1 Background — 13

2 Gene drive: impact, preconditions and uses — 15

- 2.1 Gene drive — 15
- 2.2 Preconditions for efficient use — 15
- 2.3 Possible uses — 16
- 2.4 Current situation in the Netherlands — 17
- 2.5 Unintended construction of a gene drive — 17

3 Assessment of the consequences for human health and the environment — 19

- 3.1 Introduction — 19
- 3.2 Consequences for human health and the environment: considerations — 19
- 3.3 Legal framework — 20
- 3.4 Risk assessment for contained use (CU) — 21
- 3.5 Environmental risk assessment for release into the environment (RE) — 22

4 International context — 23

- 4.1 Overview of international agendas — 23
 - 4.1.1 NAS workshops — 23
 - 4.1.2 Initial survey of EU experts — 23

5 Report and recommendations — 25

- 5.1 Report — 25
 - 5.1.1 Main points of the report — 25
 - 5.1.2 Report in detail — 25
- 5.2 Advice — 26
- 5.3 Recommendations — 27
 - 5.3.1 Research recommendations — 27
 - 5.3.2 Other recommendations — 27

6 Other points of concern — 29

7 References — 31

Summary

In August 2015, 26 scientists published a letter in *Science* calling for safety measures for the use of a genetic technique called 'gene drive'. A gene drive is a genetic trait that can switch off or change an existing trait in a population or add a new trait to the DNA of an organism. This trait spreads rapidly – and possibly irreversibly – throughout an entire population of an organism.

An organism with a gene drive is a genetically modified organism (GMO); in the Netherlands GMOs are subject to the Genetically Modified Organisms Decree 2013 (*Besluit genetisch gemodificeerde organismen milieubeheer 2013*, GMO Decree). Under this Decree, activities involving GMOs must be preceded by a risk assessment. The possible risks for human health and the environment from GMOs are assessed in the Netherlands using methodologies laid down in the GMO Decree.

A recent example (November 2015) of a gene drive application is a malaria mosquito that can no longer transmit parasites. A gene drive enables this trait to spread very rapidly throughout the mosquito population, making malaria easier to control. In theory, this principle can be applied to any trait.

Problem definition

In this policy report, RIVM looks at the environmental safety aspects of a gene drive. It examines whether the current method of assessing environmental safety aspects of GMOs adequately covers assessments of organisms with a gene drive.

Two aspects for concern have emerged from this exercise:

- The combination of the underlying traits of the gene drive – rapid, possibly irreversible and population-wide – means that the possible impact of the gene drive on human health and the environment cannot, or cannot fully, be assessed within the current methods of risk assessment.
- Unintended use of certain genetic modification technologies may occasionally give rise to a gene drive and may therefore possibly lead to an unintended and undesirable impact on human health and the environment.

Legal context

In the Netherlands, working with GMOs is governed by two statutory regimes: 'contained use' (CU) for working in a confined space and 'release into the environment' (RE) for the deliberate release of GMOs into the environment. All GMO activities under RE are subject to authorisation. With regard to CU, some activities involving GMOs are subject to authorisation while some only require notification. In the latter case, which concerns organisms without pathogenic traits, not all activities are subject to notification. It is therefore possible that activities involving organisms with a gene drive may not be carried out safely. Under the notification procedure these activities may not be visible to the authorising body.

General requirements and applications

A gene drive is only effective in organisms that reproduce sexually. Expected uses of gene drives are geared towards bringing about changes in the entire population. The most obvious use of gene drives is for organisms with a short generation time, since the generation time is the factor that determines the speed at which the trait is passed on in the population.

Initially, gene drives are most likely to be used in fundamental scientific research involving microorganisms such as yeast, and larger organisms such as insects and mice. Their use is less likely in mammals with a long generation time because of the long time needed to achieve a population-wide effect.

In the Netherlands, RIVM's GMO Office – the body responsible for processing applications to authorise activities involving GMOs – has not received (nor is it aware of) any notifications of research involving the deliberate use of a gene drive.

Safety assessment

RIVM notes that the GMO Decree permits the assessment of all aspects relating to risks to human health and the environment. The current legislation therefore offers sufficient options for assessing organisms with a gene drive. However, RIVM also notes that

- the current method of risk assessment used for activities under CU – in particular the classification of activities – is not appropriate for assessing organisms with a gene drive;
- additional knowledge and information is needed for activities under RE in order to be able to effectively assess the potential environmental risks of organisms with a gene drive.

Advice

Firstly, in light of the potential scope and, therefore, the potential severity of the consequences for human health and the environment, RIVM recommends bringing all CU-related uses of gene drives explicitly within the authorisation requirement enshrined in the GMO Decree.

This will mean that both CU- and RE-related uses of gene drives will only be authorised if a) sufficient information is available to allow the risk to be assessed; and b) the results of a risk assessment carried out on the basis of methodology and knowledge currently available reveal only a negligible risk.

An assessment can then be made on a case-by-case basis as to what the risks of the specific activities are and whether or not they can be authorised. Based on such applications, experience will be acquired in risk assessments for organisms with a gene drive. In this way the necessary measures – such as measures for safe working and containment regimes – can be tailored to these GMOs.

Secondly, RIVM recommends that conditions be laid down in the GMO Decree for the use of specific genetic techniques with the aim of preventing the unintentional creation of constructs with a gene drive. RIVM also recommends a number of avenues for further research.

Thirdly, RIVM considers it necessary to inform potential authorisation applicants about the options and possible harmful consequences of deliberate or unintended uses of gene drives.

Fourthly, RIVM emphasises the international dimensions of the current legal frameworks (global and European) and the fact that the potential consequences for human health and the environment can spread beyond national borders. An initial analysis of the situation has revealed that concerns about gene drive are already being discussed on a scientific and policy level in countries such as the USA and in a number of EU member states.

RIVM therefore recommends that this topic be debated internationally, also at the EU level, and that research be carried out into the likely uses of gene drives, into the knowledge that will be needed to assess these uses properly, and into possible control measures.

Finally, RIVM points out that there have been calls for a societal debate on the desirability of certain uses of gene drives and highlights the potential for deliberately using gene drives for harmful purposes.

1 Background

In early August 2015, 26 scientists published a letter in *Science* [1] calling on governments to introduce uniform safety measures for the use of gene drives. They also called for a public debate on the desirability of using gene drives. Shortly thereafter, in a Dutch radio interview, two Dutch scientists [2] called on the Dutch government to place the subject on the biotechnology agenda and initiate a debate on the advantages and disadvantages of this technique.

RIVM has analysed developments in the field of gene drives and their potential consequences for human health and the environment, resulting in this policy report. In compiling this report, RIVM used the following sources of information:

- the available scientific literature on gene drives;
- Dutch and European legislation on genetically modified organisms (GMOs);
- RIVM experts' own knowledge of environmental and other risk assessments of GMOs;
- information from the Dutch database of GMO authorisations; and
- information from Dutch and international experts, including risk assessors not employed by RIVM.

Scope and structure of this document

This policy report is restricted to the environmental safety aspects of a gene drive. In this context, RIVM examined whether the current method of assessing environmental safety aspects in GMOs adequately covers assessments of organisms with a gene drive, both in respect of contained use (CU) and release into the environment (RE).

Chapter 2 provides a description of the impact, preconditions and uses of gene drives. Chapter 3 looks at how the consequences for human health and the environment are assessed. Chapter 4 provides a brief overview of the developments around gene drives in the international policy context. Chapter 5 provides an overview of the problem areas identified and sets out RIVM's recommendations, and Chapter 6 describes a number of other points of concern in respect of gene drives.

2 Gene drive: impact, preconditions and uses

2.1 Gene drive

Developments in modern biotechnology are taking place at an ever increasing pace and are reaching maturity ever more rapidly. This is resulting in new uses, which in some cases raise new concerns about the risks to human health and the environment. The development of organisms with a gene drive is a recent example of this.

A gene drive can insert a new trait irreversibly into an entire population of an organism. A gene drive is a genetic trait that copies itself into the DNA of an organism together with a new trait. The gene drive passes on both the new trait and the 'copy trait' to all offspring, which in turn pass the gene drive on to their offspring, with the result that the new trait is passed on at a higher frequency than that normally seen in 'normal' inheritance (see Figure 1). A gene drive can therefore cause a new trait to spread rapidly in a population, altering that population irreversibly.

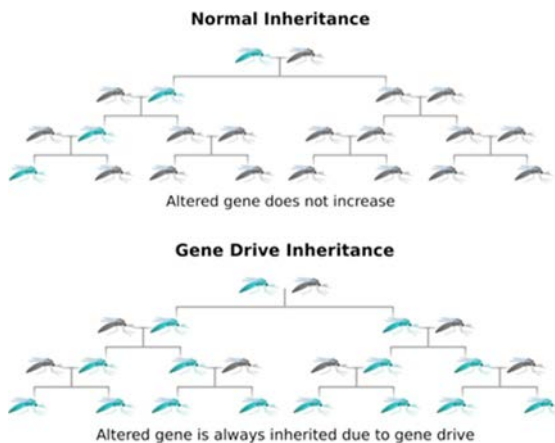


Figure 1: Normal inheritance and inheritance with gene drive. The parent with the new trait is shown in blue. In 'normal' (Mendelian) inheritance of this trait, the percentage of offspring with this trait becomes smaller after every generation. If this new trait is introduced with a gene drive, it is spread throughout the whole population within a few generations. Source: [3].

2.2 Preconditions for efficient use

The use of a gene drive in an organism is intended to rapidly bring about a population-wide effect. The use of this technology is subject to a number of preconditions.

- *Sexual reproduction*
The power of gene drive is that it passes on a trait to the offspring at a higher frequency. This effect only works in organisms that reproduce sexually. When used in organisms that reproduce in another way, such as bacteria and viruses, gene drives do not result in the new trait being passed on at a higher frequency.

- *Short generation time*
The use of gene drive is intended to bring about a rapid change in a population. For organisms with a relatively short generation time, such as mosquitoes or mice, its use will lead to an effect at the population level relatively quickly. Although gene drive can also be used in organisms with a long generation time, the effect on the entire population is much more gradual because the trait takes longer to spread through the population.
- *Homologous recombination*
The technique of inserting a gene drive uses a certain type of internal DNA repair in the cell: homologous recombination. This process is not equally efficient in all organisms. Gene drive therefore only appears to be effective in organisms in which homologous recombination can take place efficiently.

This means that if the technology is used to bring about a rapid, population-wide effect, only a small number of organisms seem to be candidate for the use of gene drive. In the laboratory setting in which gene drive is currently being tested, this might include eukaryotic microorganisms such as yeast; insects (fruit flies, mosquitoes); the model organism *Caenorhabditis elegans* (nematode); and mammals that reproduce rapidly, such as mice. To date, no literature on the use of gene drives in plants is available.

Furthermore, stable, long-term inheritance of a gene drive over several generations has not yet been demonstrated; a potential problem is that a gene drive is still subject to mutations and may therefore become ineffective.

In the USA, proof-of-principle studies are currently being carried out on gene drives in yeast [4] and *Drosophila* (fruit fly) [5]. In addition, a malaria mosquito with a gene drive was developed very recently [6]. The mosquito was altered in such a way that it could no longer pass on malaria parasites.

2.3 Possible uses

Gene drives are used to bring about a rapid alteration in an entire population. This alteration may involve switching off, altering or adding a trait in a population. Various examples of potential uses have been cited in the scientific literature [7].

A gene drive could, for example, be used to switch off a trait that changes the fitness of a harmful organism. For example, a resistance gene in a plague insect could be switched off to make the insect vulnerable to a natural toxin (e.g. Bt toxin). Another example is reducing the fitness of invasive species by switching off one or more essential traits, thus reducing their chances of survival.

Insertion of a new trait with a gene drive could be used to make populations of beneficial insects resistant to pesticides. But gene drive technology could also be used for fundamental research in areas such as the use of mice as animal models for studies on human diseases.

Because gene drive passes on the desired test trait to all the animals rather than only to some, fewer laboratory animals would be needed.

In the short term, organisms with a gene drive are only likely to be used for fundamental scientific research in the laboratory. However, developments are taking place rapidly, as can be seen in the example of the malaria mosquito mentioned above.

Example 1

The fruit fly is an example of a species that can be altered rapidly with gene drive. Fruit flies can be modified with a gene that determines eye colour. If this trait is inserted in the fruit fly via a gene drive and the fly is released into the environment, this trait can be rapidly transferred to fruit flies that exist naturally in the ecosystem. As a result, the entire population of fruit flies may acquire this new eye colour.

Example 2

Gene drive can be used in cultures of mammalian cells without the risk of rapid spread in a population. Like bacteria, these cells multiply without sexual reproduction. This means that a genetic modification brought about by inserting a gene drive in mammalian cell cultures will not lead to more rapid spread in mammals.

2.4 Current situation in the Netherlands

Based on the applications for authorisation submitted in the Netherlands, no activities with gene drive are known in the Netherlands. For some uses that fall within contained use, notification is sufficient. However, it is not inconceivable that a gene drive could be constructed as a result of the combination of these sub-uses. This would not come to the attention of the authorising body.

In the Netherlands there are currently no applications for authorisation under either contained use or release into the environment in which gene drive is explicitly referred to as a use. But in light of the opportunities for using gene drive in fundamental research referred to above, it is quite conceivable that applications will soon be made for using gene drive under contained use in the Netherlands.

The use of gene drive in organisms with the intention of deliberately releasing these organisms into the environment does not currently seem to feature in Dutch research. However, this may change in the future if, for example, gene drive can be used effectively to control insect-borne disease transmission (such as in ticks to prevent the spread of Lyme's disease).

2.5 Unintended construction of a gene drive

A gene drive consists of two separate elements, each with its own functionality, which integrate together in the DNA when used as a gene drive. However, these separate elements are also frequently used in techniques such as genome editing, i.e. bringing about specific changes in an organism's DNA. In genome editing, both elements are generally inserted in the cell temporarily in order to bring about the genetic modification, after which they disappear from the cell again. If both

elements are inserted in the cell on the same DNA construct, it is possible that this combination of elements will in incidental cases become part of the DNA of the host via homologous recombination. As a result, there is a slight risk that this could lead to a gene drive. Users of these genetic elements must therefore be aware of the possibilities and potentially harmful consequences of deliberate or unintended use of gene drives.

3 Assessment of the consequences for human health and the environment

3.1 Introduction

Organisms with a gene drive are genetically modified organisms. Working with organisms with a gene drive therefore falls within the scope of the GMO Decree [8]. This decree requires a risk assessment to be performed prior to any use of a GMO. It also specifies how this risk assessment is to be carried out.

For contained use, the purpose of the risk assessment for GMOs is to determine the containment measures under which the activities can take place. For release into the environment, conversely, the explicit aim is to release the GMOs into the environment. The environmental risk assessment is therefore designed to ensure that this release takes place without inflicting damage on the environment.

In the following subsections we first describe a number of specific aspects of an organism with a gene drive that are relevant to a risk assessment (3.2). This is followed by a description of the legal framework (3.3) and a more detailed analysis of the assessment methodology and its suitability for assessing organisms with a gene drive under contained use (3.4) and for release into the environment (3.5).

3.2 Consequences for human health and the environment: considerations

If released into the environment, an organism that has been modified with gene drive technology differs in three respects from other GMOs regarding its potential impact on human health and the environment.

- *Potential scope of population change*
An organism with a gene drive potentially has the capacity to alter the entire population. Such an organism can therefore have an effect at the population level. The most extreme consequence of this is 'replacement' of an existing population of an organism with a 'new', altered population. Without a gene drive, a trait takes much longer to spread through a population and, in many cases, disappears again from the population.
- *Rate of population change*
By definition, every offspring of an organism with a gene drive will have the gene drive trait. The trait therefore spreads through the population much more rapidly than if it had been introduced without a gene drive mechanism. Obviously, the rate at which population change takes place also depends on other aspects such as the generation time (the shorter the time, the more rapid the spread) and the number of organisms with a gene drive introduced into the population.

- *Irreversibility of the population change*
Although it cannot be stated with any certainty, it is likely that a change brought about in a population with a gene drive will be permanent. There is no natural mechanism – such as natural selection – that will eliminate the inserted gene drive trait. This differs from most GMOs without a gene drive: a trait for which no selection takes place will eventually disappear from a population because the organism gains no benefit from it. Research is being done [7] into ways of reversing the effect of a gene drive by, for example, inserting a second gene drive. However, the genetic elements of the gene drive would still remain in the population, potentially resulting in the accumulation of foreign genetic material.

A consequence of the above is that all potential effects on human health and the environment must be considered at the level of the entire population. This also means that the consequences of an entire population change, on the ecosystem for example, must be examined.

3.3 Legal framework

In the Netherlands, authorisation and use of genetically modified organisms are governed by the GMO Decree [8]. This decree is to a large extent based on European legislation. The European legislation on this topic comprises two directives.

- *Contained use (CU) - Directive 2009/41/EC*
Directive 2009/41/EC [9] relates to the contained use of genetically modified microorganisms and is intended for activities with genetically modified microorganisms (GMMs) involving containment measures to prevent the GMMs from coming into contact with humans and the environment. Besides implementing the EU directive, the Dutch GMO Decree also lays down additional rules for contained-use activities with organisms other than microorganisms, such as insects, animals and plants.
- *Release into the environment (RE) - Directive 2001/18/EC*
Directive 2001/18/EC [10], which relates to deliberate release into the environment, applies to all genetically modified organisms. This EU directive is also implemented in the GMO Decree.

With regard to both CU and RE, the definition of a GMO is included in Article 1.1 of the GMO Decree. Organisms with a gene drive conform to this definition and are therefore subject to the provisions of the decree.

In brief, the decree stipulates that:
a genetically modified organism is an organism whose genetic material has been changed by means of a method that does not occur naturally. If this change has taken place with the help of recombinant nucleic acid technologies, the organism is always classified as genetically modified. An organism with a gene drive conforms to this description.

The decree stipulates that all activities involving GMOs are subject to authorisation or, for some cases that fall under CU, to notification. As part of the authorisation or notification procedure, a risk assessment is required to identify the risks to human health and the environment and, if necessary, to determine measures for preventing or containing these risks. The risk assessment differs depending on whether the use falls under CU or RE. The reason for this is that, as pointed out earlier, the starting point for CU is that the GMO will remain in a contained environment, while RE involves deliberate release into the environment.

3.4 Risk assessment for contained use (CU)

For contained use activities, an assessment is made as to whether the containment measures for the GMO activity concerned are sufficient to prevent contact with humans and the environment as far as possible, and to keep the risks to a negligible level. Most CU activities involve microorganisms, for which the rules and assessment system are laid down in EU Directive 2009/41/EC. The directive does not apply to organisms other than microorganisms, such as insects, plants or laboratory animals such as mice; in the Netherlands separate national regulations are laid down in the GMO Decree.

Current legislation permits an assessment of all aspects relating to the risks to human health and the environment. In practical terms, however, this assessment has only been introduced for CU to a limited extent and the risks are primarily assessed on the basis of the pathogenicity of the GMO to humans, animals and plants.

For organisms with a gene drive used in CU activities, RIVM notes the following:

- Current legislation provides sufficient opportunities to implement an effective risk assessment methodology.
- The risk assessment methodology for CU laid down in European legislation relates to microorganisms and is organised on the basis of pathogenicity classes. This approach is not particularly suitable for assessing the consequences of activities with organisms with a gene drive, since these organisms may not be pathogenic. However, since they have a new trait with a high probability of spreading, the consequences of releasing the organism into the environment need to be assessed differently from those relating to pathogenicity.
- A national methodology is available for risk assessment and classification of CU activities involving organisms other than microorganisms. However, the classification of activities in various containment regimes is not designed for activities with a gene drive because, being based on EU legislation, it is mainly geared towards pathogenicity.

Conclusion

The directive and the current legal framework offer sufficient options to implement a risk assessment for organisms with a gene drive. However, the current classification of activities with GMOs is not suitable for organisms with a gene drive.

3.5 Environmental risk assessment for release into the environment (RE)

In the case of RE, the assessment focuses on whether the GMO poses only a negligible risk to the environment. Any deliberate release of a GMO into the environment is subject to authorisation and must always be preceded by an environmental risk assessment. The assessment framework and method are laid down in EU Directive 2001/18/EC, which is implemented in the GMO Decree.

Release of GMOS into the environment takes place according to the 'step-by-step' principle. This means that, following an environmental risk assessment, humans and the environment are exposed to the GMO incrementally, provided the previous step reveals that it can pose only a negligible risk. During each step, data is collected that is necessary for assessing the environmental risk for the next step.

Incremental release of organisms with a gene drive into the environment is more complicated due to the risk of population-wide spread. However, alternative approaches are possible, for example by studying the organism in an environment containing no related species. Another possible option is to investigate the environmental effects of the desired trait in the absence of the 'copy element' of the gene drive.

With regard to organisms with a gene drive released into the environment, RIVM notes the following:

- Current legislation offers a method for carrying out a case-by-case assessment of potential risks to human health and the environment of all GMOs, i.e. including organisms with a gene drive. Current legislation therefore provides sufficient opportunities to put an effective risk assessment in place.
- Until now, the environmental risk assessment of organisms released into the environment has not considered the aspect of irreversible changes at the level of the entire population. Therefore, in order to put an adequate environmental risk assessment in place for organisms with a gene drive, additional knowledge and information are needed – depending on the application – to assess changes at the population level.
- While the step-by-step principle that guides the incremental release of a GMO into the environment is probably still usable as a generic principle, for organisms with a gene drive it calls for a different approach that takes account of the specific environmental consequences of this technology.

Conclusion

Current legislation provides sufficient opportunities to implement an effective environmental risk assessment methodology. The 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.

4 International context

4.1 Overview of international agendas

International scientific interest in gene drive technology has increased significantly since the summer of 2015, as have the associated concerns. But growing attention is also being paid to the topic in the opinion-leading press and among policy-makers. Informal contact between RIVM and experts in the Netherlands and abroad has revealed that these experts are aware of the problems and that the topic is being addressed both on a scientific and a policy level.

4.1.1 *NAS workshops*

The US National Academies of Sciences, Engineering, and Medicine (NAS) have scheduled a series of seven events on gene drives [11]. Following these events, a report will be published on the science, ethics and governance of gene drive research.

At a workshop in Washington, USA on 1 November 2015, it became clear that there was still much confusion surrounding gene drives on the part of scientists as well as policy-makers. A brief news report published on the outcomes of this workshop stated that few if any organisms are so well characterised that we can predict the ecological effects of changes to or the disappearance of a population as a result of a gene drive. In addition, no effective safe strategy has been developed to stop the spread of a gene drive. The report also mentioned that it would be useful to put in place international agreements on how to handle gene drives, since organisms with gene drives released into the environment can cross national borders.

4.1.2 *Initial survey of EU experts*

In an informal survey of European experts on the environmental safety of GMOs, RIVM asked such experts whether they have information on the use of gene drives in their country and whether, and if so how, the use of gene drives is regulated in their country. Experts from eight member states responded.

This survey revealed that not all experts know whether there is research being done into gene drives in their country. One expert reported that in the context of contained use, research is being done into gene drives in yeast, fruit flies and mosquitoes. Another expert suspected that people were working with gene drives, while other experts had not received any applications or had any indications that work was being done with gene drives.

Experts from various EU member states reported that the first activities with gene drives under the contained use regulations are expected. Several experts did state that it was not yet clear under which containment measures organisms with a gene drive should be used, but that a case-by-case approach was currently considered to be the most suitable for arriving at a suitable classification.

One of the experts mentioned that work with organisms with gene drives may be going on unnoticed. This is because while gene drive components have been reported through notification, there is no insight into the ultimate use.

Discussions of the potentially harmful environmental impact of releasing an organism with a gene drive into the environment, whether deliberate or accidental, have not yet begun or are in the start-up phase in the surveyed EU member states. Our enquiries about the main potential risks that have been identified and about the uncertainties about a release into the environment produced a similar picture to that described in this report. In addition to the potential risks that are similar to those of the current generation of GMOs, the main aspects that set a gene drive apart are the rapidly spreading, permanent (irreversible) changes they can bring about in entire populations, even if the introduced trait does not entail a selective advantage.

All the surveyed experts acknowledge the potential cross-border nature of organisms with gene drives and the possible impact on human health and the environment. Like the experts in the Netherlands, they would like to see a discussion take place on this subject at the European and international level and are in need of an internationally harmonised strategy.

5 Report and recommendations

5.1 Report

5.1.1 *Main points of the report*

Early identification of new risks is one of the starting points in the assessment framework for safety and risk policy[12]. This report elaborates on this aspect and takes an initial step in this direction.

Developments in modern biotechnology are taking place at an ever increasing pace and are reaching maturity ever more rapidly. The development of organisms with a gene drive is an example of this.

An organism with a gene drive is a genetically modified organism. A gene drive is a genetic trait that can switch off or change an existing trait in a population or add a new trait to the DNA of an organism. This trait spreads rapidly and irreversibly throughout an entire population of an organism.

According to an analysis of the way in which risk assessment currently takes place, this method gives rise to concern in relation to organisms with a gene drive for two reasons:

- The combination of the underlying traits of the gene drive – rapid, irreversible and population-wide – means that the possible effects of the gene drive on human health and the environment cannot be assessed effectively with the current methods of risk assessment.
- Poorly conceived uses of certain genetic modification techniques may sporadically result in a gene drive and could therefore lead to unintended and undesirable effects for human health and the environment.

In light of the potential scope and, therefore, the potential severity of the consequences for human health and the environment, RIVM recommends bringing all CU-related uses of gene drives explicitly within the authorisation requirement under the GMO Decree.

This means that both CU- and RE-related uses of gene drives will be eligible for authorisation only if a) sufficient information is available to allow the risk to be assessed, and b) the risk assessment performed on the basis of the current methodology and knowledge available reveals a negligible risk.

As knowledge and insight progress, the assessment method can be adapted appropriately with additional categories of containment tailored specifically to organisms with a gene drive.

5.1.2 *Report in detail*

RIVM investigated whether the current method of assessing the risks of GMOs for human health and the environment is suitable for organisms with a gene drive. It reached the following conclusions:

- The GMO Decree and the underlying EU directives on contained use (Directive 2009/41/EC) and release into the environment (Directive 2001/18/EC) of genetically modified organisms are applicable and offer a suitable basis for assessing the risks of organisms with a gene drive to human health and the environment.
- The method of risk assessment or the way in which it is applied is not always adequate for effectively assessing the risk of an organism with a gene drive to human health and the environment.
- Current risk assessment methodology does not always enable an opinion to be issued on the risk to human health and the environment. There are two reasons for this, depending on whether the use is contained (CU) or the organism is to be released into the environment (RE):
 - for CU the main reason is that the assessment methodology uses a classification into risk categories based on pathogenicity classes that has nothing to do with assessing the impact of an organism with a gene drive;
 - for RE this is primarily due to the absence of the right type knowledge and information needed to assess impact at the population level.
- The genetic elements needed to construct a gene drive are already widely used in genome editing activities. If these elements are inserted into the cell on the same DNA construct, it is possible that this combination of elements will in incidental cases become part of the DNA, thereby creating a potential gene drive.
- In the Netherlands, activities with GMOs are subject to authorisation or, in the case of the lowest risk categories under contained use, notification. In the latter case, moreover, not all activities need to be notified. It is therefore possible that activities involving organisms with a gene drive could be carried out at the lowest level without it being clear whether the safety level is appropriate for these gene drive uses, and without notification, and therefore not visible to the authorising body.

5.2 Advice

On the basis of the above, RIVM therefore offers the following advice:

It is essential that researchers are aware of the potential risks and consequences of working with organisms with a gene drive. It is also important to have a picture of current developments and possible uses of organisms with a gene drive in the Netherlands. RIVM therefore advises the Ministry of Infrastructure and the Environment to

- explicitly make *all* uses of gene drive subject to authorisation with the aim of
 - assessing the environmental risks of all activities with organisms with a gene drive and determining on a case-by-case basis whether authorisation can be granted;
 - further developing the risk assessment in relation to the aspects that are relevant to a gene drive, based on

- maintaining permanent scrutiny on developments and uses of organisms with a gene drive.
- prevent the unintentional creation of a gene drive by laying down conditions in the GMO Decree for the use of specific genetic elements.

5.3 Recommendations

5.3.1 *Research recommendations*

The knowledge gaps about both CU and RE must be filled in to ensure that the methodology and its application are suitable for assessing the environmental risks of organisms with a gene drive. In broad terms, the research should cover the following areas:

In terms of CU:

- Which scientific deliberations play a role in setting up an assessment methodology for organisms with a gene drive?
- Which containment measures are suitable and effective for organisms with a gene drive and how can they be established?
- What additional information and knowledge is needed to conduct an effective risk assessment and draw up a management strategy?
- To what extent is this kind of knowledge already available or to what extent is further development required?
- Can we further specify the likelihood of a gene drive being created unintentionally and when this could give rise to a risk?

In terms of RE:

- What additional knowledge and information is needed to enable an effective environmental risk assessment to be drawn up?
- To what extent is this kind of knowledge already available or to what extent is further development required?
- How can the step-by-step principle be implemented in a manner that is appropriate for organisms with a gene drive?

5.3.2 *Other recommendations*

RIVM emphasises the need to place this matter on the international scientific and policy agenda and to obtain international agreement on the topic of gene drives. Since the basis of the legislation lies in Europe, it is essential that this issue is placed on the agenda and discussed at the European level in order to enable it to be developed in greater depth and enable knowledge to be acquired. This applies both to the details of the risk assessment for contained use and for release into the environment.

In addition, RIVM sees a need to inform current and potential authorisation applicants promptly about the options and possible harmful consequences of the use of gene drives; to draw their attention to the need for control measures; and to inform them of the need to avoid unintended gene drives, and only to use deliberate gene drives if safety can be guaranteed.

6 Other points of concern

Societal debate

In the article published in *Science* in early August 2015 [1], the authors not only called for uniform safety measures but also for a public discussion on the desirability of the use of gene drives. They stressed that the potential of a gene drive to spread a particular trait irreversibly, rapidly and efficiently throughout an entire population also entails ethical issues.

For example, they reported that some uses of a gene drive, such as the elimination of malaria-transmitting mosquitoes, will raise concerns as to the societal desirability of using gene drives in this way. Moreover, potential malicious misuse (see below) and ecological implications could become part of a broader discussion.

Other legislation

Besides the GMO Decree, other legislation applies to genetically modified organisms. Whether this legislation is also suitable for the use and control of organisms with a gene drive needs to be examined in greater depth. This concerns legislation which governs the transportation of GMOs and the specific rules on storage and destruction of GMOs.

Malicious use

A gene drive can also be used maliciously to cause the rapid spread in a population of a genetic product with a harmful effect. The example cited in the literature is the insertion of a gene for a toxin into a human blood-sucking insect, whereby a bite transmits the toxin and causes blood poisoning. It is also important to include this aspect in the discussions on gene drives.

Do-It-Yourself (DIY) biology

It is relatively simple to bring about a targeted genetic modification with the use of the genetic elements CRISPR and Cas9. These elements are currently being offered in kit form to the DIY biology community [13]. Since a gene drive can be made using these CRISPR-Cas9 elements, there are concerns over the potential for inexpert use of these elements.

The above points of concern can be incorporated into subsequent steps in the policy phase consistent with a deliberate approach to safety [12]. This process can also include an assessment of how safety and innovation can strengthen each other.

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