



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

**Risk assessment method for activities
involving organisms with a gene drive
under contained use**

RIVM Letter report 2018-0090
C.J.B. van der Vlugt et al.



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Colophon

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Synopsis

Risk assessment method for activities involving organisms with a gene drive under contained use

A gene drive is a genetic trait that is passed on to almost all the offspring. As a result, a gene drive can spread quickly and permanently through an entire population. Gene drives can occur naturally or may be created in laboratories by genetic modification. The use of gene drives investigated here refers to the latter form. The majority of developments are still in the R&D phase and it will be a number of years yet before they can be used in practice. In the meantime, it is important to be able to determine how to use gene drives safely in laboratories and animal centres ("contained use").

In 2015 RIVM warned that potentially harmful effects of organisms with a gene drive on humans and on the environment cannot be assessed (or not sufficiently assessed) with the method used until then for risk assessment for contained use. Follow-up research has shown that the method does offer leads that will permit proper risk assessment, and those leads have now been examined in detail. It was also observed that the risk assessment must be based on other harmful effects than the ones that have been researched so far. In addition, measures were proposed that could minimize the potential risks of contained use, such as an extra safety cage or airlock in the laboratory. The measures vary depending on the type of organisms; they are for instance different for arthropods than for rodents.

RIVM stresses the importance of remaining properly aligned with scientific developments in gene drive applications. Given the potential for cross-border effects as well, it is also necessary to enter into discussions in international and European context about the risk assessment method for contained use of gene drives. For this research RIVM has been collaborated with knowledge institutes in England, Belgium and Germany.

In the legislative amendment to the GMO Regulation in July 2016, the Ministry of Infrastructure and Water Management decreed that permits must be requested for all applications using gene drives. The rules, with the risk assessment method described here, offer sufficient flexibility to permit the technique when the risks for humans and the environment are negligible.

Keywords: gene drive, CRISPR/Cas9, genetically modified organism, contained use, risk assessment, risk management.

Publiekssamenvatting

Risicobeoordelingsmethode voor organismen met een 'gene drive' toegepast onder ingeperkt gebruik

Een gene drive is een genetische eigenschap die nagenoeg aan alle nakomelingen kan worden doorgegeven. Hierdoor kan een gene drive zich snel en blijvend in een hele populatie verspreiden. Gene drives kunnen van nature voorkomen of door genetische modificatie in laboratoria worden gemaakt. Het hier onderzochte gebruik van een gene drive betreft deze laatste vorm. De meeste ontwikkelingen zitten nog in de onderzoeksfase en het zal nog jaren duren voordat ze in de praktijk kunnen worden gebruikt. Ondertussen is het van belang om te kunnen bepalen hoe in de onderzoeksfase in laboratoria en dierverspreiden ('ingerperkt gebruik') veilig met gene drives kan worden gewerkt.

Het RIVM signaleerde in 2015 dat mogelijk schadelijke effecten van organismen met een gene drive op mens en milieu niet of onvoldoende beoordeeld kunnen worden met de tot dan toe gebruikte methode van risicobeoordeling voor ingeperkt gebruik. Uit vervolgonderzoek blijkt dat de methode wel aanknopingspunten biedt voor een goede risicobeoordeling, en die zijn nu uitgewerkt. Ook blijkt dat de risicobeoordeling op basis van andere schadelijke effecten moet worden uitgevoerd dan waarmee tot nu toe ervaring was opgedaan. Daarnaast worden maatregelen voorgesteld die de mogelijke risico's bij ingeperkt gebruik tot een minimum kunnen beperken, zoals een extra veiligheidskooi of sluis in het laboratorium. Deze maatregelen verschillen per groep van organismen; voor insecten zijn ze bijvoorbeeld anders dan voor knaagdieren.

Het RIVM onderstreept het belang om goed aangesloten te blijven bij de wetenschappelijke ontwikkelingen op het gebied van gene drive-toepassingen. Ook is het, mede gezien de mogelijk grensoverschrijdende effecten, nodig om in internationaal en in Europees verband in gesprek te gaan over de risicobeoordelingsmethode voor ingeperkt gebruik van gene drives. Het RIVM werkte voor dit onderzoek dan ook samen met kennisinstellingen uit Engeland, België en Duitsland.

Met de wetwijziging van de Regeling ggo in juli 2016 heeft het ministerie van IenW bepaald dat voor alle toepassingen met gene drives een vergunning moet worden aangevraagd. De regeling biedt met de hier beschreven risicobeoordelingsmethode voldoende flexibiliteit om de technologie toe te staan wanneer de risico's voor mens en milieu verwaarloosbaar zijn.

Kernwoorden: gene drive, CRISPR/Cas9, genetisch gemodificeerd organisme, ingeperkt gebruik, risicobeoordelingsmethode, risicomangement.

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Summary

A gene drive ensures transmission of a modified or new genetic trait to nearly all offspring, instead to only some offspring. This allows this trait to spread permanently and quickly in an entire population. In 2015 the RIVM determined that the potential harmful effects of an organism with a gene drive under contained use cannot be assessed, or cannot be assessed adequately, using the prevailing risk assessment method for genetically modified organisms at that time.

At the request of the Ministry of Infrastructure and Water Management, RIVM conducted a study into two aspects of the situation: what is needed to 1) assess the risks of organisms with a gene drive, and 2) take adequate risk control measures, nationally and internationally, for the contained use of such organisms.

The study was conducted in three phases: First, interviews were conducted with research groups in this field in the Netherlands. These interviews showed that at that time there were no initiatives for these groups to use gene drive technology in their own research. In addition, it became clear that gene drives were found to be interesting only for use in natural populations and not for genetically modifying animal populations in the laboratory. Gene drive applications would therefore most likely be for fundamental research or to develop a population for deliberate release into the environment.

As a second step, European researchers, biological safety officers and risk assessors were invited to a working session on this topic. During the working session, scenarios were discussed for using gene drives in three models: yeast, mice and fruit flies. These scenarios showed that the escape of a gene drive organism from a laboratory can have unpredictable – and potentially severe – consequences for humans and the environment. Due to the spreading potential of these organisms, a gene drive can spread rapidly and permanently in a population, and may also cross national borders. In that case, the experts concluded that neighbouring countries had to be informed when an incident occurred with a potentially severe risk to humans and the environment.

In this working session, the previous conclusion of the RIVM was confirmed that the most relevant aspects for assessing the risk are the severity and potential of a gene drive spreading outside a laboratory. In the current method of risk assessment, little experience has been gained with these aspects. This is because pathogenicity (disease-causing potential) is the effect that most often needs to be assessed. The experts also concluded that the risk assessment method according to the European Directive 2009/41 on the contained use of genetically modified microorganisms offers sufficient starting points to assess organisms with a gene drive.

As a third step in the research approach, a working group of risk assessors from England, Germany, Belgium and the Netherlands was formed. This group has explored the development of a risk assessment

method for gene drive applications and the formulation of suitable control measures and possibilities for incident management during contained use.

The risk assessment method consists of three elements: 1) identification of the potentially harmful effects, 2) determination of the severity and potential that these effects can occur and 3) determination of a risk class. This report provides a step-by-step description of how this method can be used for organisms with a gene drive. It also provides two examples of a risk assessment of such an organism.

RIVM also looked at which containment measures are necessary to ensure that the risks are negligible. Gene drives can be used in organisms such as yeast, arthropods and rodents. Because the biology of these groups of organisms is different, we have proposed a specific set of containment measures for each group, along with sets of working practices and possibilities for adequate measures to be taken by the user in the event of an incident.

RIVM emphasises the importance of continuing to track developments in gene drive technology by reading the scientific literature and by connecting to the international network in this field. An enormous development in knowledge is currently taking place regarding the possibilities of using this technology in fundamental research.

RIVM also stresses the importance of European-wide agreement on the methods of risk assessment and risk management when performing activities involving organisms with a gene drive under contained use, also in view of the possible cross-border effects in case such an organism escapes from a laboratory.

Finally, the RIVM has made recommendations specifically tailored to the system in the Netherlands for authorising research with gene drive organisms under contained use. The GMO Regulation (this refers to the corresponding Dutch legislation) offers sufficient flexibility to authorise activities involving an organism with a gene drive if such activities have a negligible risk for humans and the environment.

1 Introduction

1.1 Background

In the 'Gene drives' policy report [1], RIVM made a number of recommendations to the former Ministry of Infrastructure and the Environment (IenM). One of the recommendations was to conduct further research into the risk assessment method for activities involving organisms with a gene drive under laboratory conditions, i.e. 'under contained use'. In addition, the RIVM has recommended that all applications of gene drive technology must be authorised and that the gene drive topic is prioritised in an international context.

What is a gene drive and why is this a concern?

In August 2015, a consortium of 26 scientists published a letter in *Science* [2] which called for safety measures when using a genetic technique called *gene drive*. A gene drive is a genetic trait that can disable an existing trait in a population, change it, or add a new trait to the DNA of an organism. This property spreads *rapidly* and possibly *irreversibly* in an *entire population* of an organism. In 2015 [1] the RIVM determined that the potentially harmful effects of an organism with a gene drive under contained use for humans and the environment cannot be assessed, or cannot be assessed adequately, using the prevailing risk assessment method for genetically modified organisms at that time.

In response to this RIVM policy report, the state secretary of IenM informed the Lower House of Parliament about the new biotechnological development known as 'gene drive'. In her letter [3], the state secretary indicated that the ministry would take measures in the authorisation of research involving the contained use of genetically modified organisms (GMOs) to prevent gene drive technology from being used without having established that the risks are negligible. These measures were implemented in July 2016 by an amendment to the 'Regeling genetisch gemodificeerde organismen milieubeheer 2013' (hereinafter the GMO Regulation) [4].

In addition, the state secretary stated in her letter that RIVM would be asked to gain insight into what is needed to better assess the risks of gene drive technology and to take adequate risk management measures, also in an international context. The research questions are listed in Section 1.3. The following section first discusses the specifics of the amendment to the GMO Regulation.

1.2 Explanation of the amendment to the GMO Regulation, July 2016

The Ministry of Infrastructure and the Environment (currently the Ministry of Infrastructure and Water Management – IenW) amended the GMO Regulation [4] in connection with the aforementioned RIVM report. The aim of the amendment was to ensure that gene drive technology under contained use would be subject to mandatory authorisation. The authorisation process involves assessment of the proposed activity on a case-by-case basis and specification of required containment measures. If these measures ensure a negligible risk for humans and the

environment, the activity can be authorised. This procedure ensures safety at a generic level and makes innovation possible for individual activities.

The amendment of the GMO Regulation is structured as follows. A classification article has been added to Appendix 5, which defines activities involving organisms with a gene drive and classifies them at the strictest containment level, with the associated authorisation. An applicant wishing to use organisms with the gene drive technology may request a lower containment level in accordance with Art. 2.8 of the Besluit ggo milieubeheer 2013 (hereinafter GMO Decree) [5], but only if the applicant can substantiate that the risks to humans and the environment are negligible at this lower level.

Classification Article 5.0 of Appendix 5 of the GMO Regulation [4]:

Activities with organisms capable of sexual reproduction and genetically modified with a DNA sequence encoding a sequence-specific endonuclease that can integrate at or near a position in the host genome corresponding to the cleavage site of the endonuclease. Containment category: level IV.

Explanation: an endonuclease is a protein that can cut the DNA. Sequence-specific means that this cut can occur only at a location in the genome that is specifically recognised by the endonuclease and is often unique. A well-known example of a sequence-specific endonuclease is CRISPR/Cas9, but a 'zinc-finger' nuclease is also a sequence-specific endonuclease.

The RIVM policy report [1] also stated the concern that a gene drive could be created 'unintentionally'. This concern lies in the fact that the individual genetic elements of a gene drive are commonly used as tools for genome editing at the lowest containment level in combination with non-pathogenic organisms such as rodents or arthropods. Due to injudicious use when combining these genetic elements, a gene drive could be created and used at an inadequate containment level (too low). The classification article now formulates criteria (see also [6]) that specify how the use of these elements can lead to a gene drive. By including these criteria in Appendix 5, which establishes the basis for the mandatory risk assessment, the concern that a gene drive can be created unintentionally has been alleviated.

1.3 Research questions

In its policy report [1], RIVM identified knowledge gaps in the areas of risk assessment and risk management of activities involving organisms with a gene drive under contained use.

RIVM recommendations on the risk assessment method for contained use

- which scientific considerations play a role in designing an assessment method for organisms with a gene drive;
- which containment measures are suitable and effective for organisms with a gene drive and how can this be determined;

- what additional information and knowledge is needed to perform an adequate risk assessment and formulate a risk management approach;
- to what extent is this type of knowledge already available, or does this knowledge require further development;
- what is the chance that a gene drive can be unintentionally created and when can this lead to a risk.

At the request of the Ministry of Infrastructure and Water Management, RIVM carried out a follow-up study and the recommendations were used to formulate the following research questions:

1. How can the risk assessment method be applied to the contained use of GMOs with a gene drive so that adequate risk management measures can be established?
2. What containment measures are needed to ensure that the risk of an organism with a gene drive spreading into the environment is negligible?
3. What are the possibilities for incident management?

The research results and conclusions presented in this report will clarify the extent to which knowledge and information is currently available to answer these questions (see third and fourth points of the original recommendations). This will identify areas which require additional research or additional data.

The recommendation to specify how to avoid the possibility of unintentionally creating a gene drive, has been implemented by including a specific article on classification in Appendix 5 of the GMO Regulation (see 1.2).

The third research question on incident management was added at the request of the Ministry of Infrastructure and Water Management.

1.4 Delineation

This report focuses on the risk assessment method for activities involving organisms with a gene drive under contained use. This choice is based on the fact that gene drive technology will initially be developed in laboratories; intentional release into the environment will become an issue only after this phase. In addition, much attention is already being paid to the environmental risk assessment in international bodies such as in the National Academies of Sciences, Engineering, and Medicine in the USA and in the Lorentz workshop in the Netherlands.

In this report a gene drive is defined as a gene drive technology that complies with the formulation of the classification article referred to in Section 1.2 and which is therefore generated through genetic modification.

In some applications of a gene drive, an additional genetic trait, a 'cargo gene', is added to the gene drive. The potentially harmful effects of the cargo gene must also be determined in the risk assessment. However, this report focuses on determining the potentially harmful effects of the gene drive itself.

1.5 Reading Guide

This report describes the research carried out by the National Institute for Public Health and the Environment (RIVM) into the requirements for an adequate risk assessment method and the determination of control measures of activities involving organisms with a gene drive under contained use.

Chapter 2 provides a description of the step-wise methodology for answering the research questions. Chapter 3 summarises the results of the knowledge and information collection process, after which Chapter 4 discusses the answers to the research questions and Chapter 5 presents the conclusions and recommendations. The report concludes with Chapter 6 on the transposition of these research results to the Dutch authorisation system for activities under contained use.

2 Method

2.1 Addressing the research questions

To prepare for answering the research questions listed in Section 1.3, two initiatives (see 2.2 and 2.3) were implemented to contact a wide range of experts with the aim of exchanging knowledge and gathering information. To address the research questions, a working group consisting of European risk assessors was created (see 2.4).

2.2 Interviews with actors in the corresponding research field in the Netherlands

To explore the possibilities of gene drive technology and its desirability in research in the Netherlands, in the autumn of 2016 telephone interviews were conducted with a number of biological safety officers (BSOs), researchers using site-specific nucleases such as CRISPR/Cas9, and head researchers at laboratory animal facilities in the Netherlands (potential users of this technology). The interviews focused on the possibilities they saw for using gene drive technology in their own research field.

2.3 Working session with experts

In January 2017, RIVM organized a one-day working session in Utrecht with risk assessors familiar with contained use from a number of European countries, BSOs and scientists (in the Netherlands and abroad) in the fields of CRISPR/Cas9 and gene drive technology. The aim was to explore the level of concern about gene drive technology in the various European countries and to identify the possibilities and difficulties for assessing the risks of a gene drive.

2.4 EU risk assessors working group

Based on this working session, a working group of risk assessors from the Netherlands, Belgium, Germany and the United Kingdom was formed to investigate the development of a risk assessment method for activities with gene drives, the establishment of suitable control measures and the possibilities for incident management. This resulted in the formulation of the three research questions (see 1.4).

In view of the conclusion from the working session (see 3.2) that the risk assessment method according to the European Directive 2009/41/EC¹ [7] provides sufficient guidelines for the assessment of organisms with a gene drive, this method was taken as the point of departure.

The results of this working group were published in the journal *Applied Biosafety* [8] and form the basis of Chapter 4.

¹ As used in the present report 'the Directive' refers to European Directive 2009/41/EC on the contained use of genetically modified microorganisms.

3 Outcomes of the interviews and working session

3.1 Reconnaissance of the corresponding research field in the Netherlands

The interviewees (in the autumn of 2016) were unanimous in stating that there was no immediate interest in including gene drive technology in their research. They stated that using a gene drive to develop animal models for applications such as biomedical research ('lab populations') was not attractive because the current transgenesis techniques are satisfactory. Moreover, using a gene drive for animal models would have disadvantages because, in addition to the desired mutation, the endonuclease is also present. The researchers indicated that using a gene drive in animals is attractive only if the aim is to genetically modify a natural population, not a lab population.

Based on of these interviews, RIVM concluded that there was little interest in the Netherlands for working with gene drive technology in the short term. In any case, very few requests for authorisation of gene drive applications can be expected in the near future.

In the autumn of 2017, a biological safety officer contacted the RIVM because a research group in their organisation was interested in using gene drive technology. The procedure for requesting an authorisation was discussed and the information that must be provided to substantiate the request was specified. Whether an actual request for authorisation will ensue appears to depend on the decision about funding the research proposal (expected in mid-2018).

3.2 European working session

A number of experts confirmed the earlier conclusion of RIVM [1] that the gene drive technology could be classified at containment level I on the basis of the current method of risk assessment, so that activities could be started immediately without the assessment being reviewed by the government. Inadequate containment can lead to potentially severe risks for humans and the environment in the event of an unintentional release from the laboratory. A gene drive can spread rapidly and permanently in a population, which means that it could also spread across national borders. In that case, the experts concluded that neighbouring countries had to be informed in case of an incident with a potentially severe risk to humans and the environment.

At the time the working session was held, both Germany and England had already distributed recommendations or guidance materials to inform researchers that GMOs with a gene drive have an increased risk of spreading outside the laboratory. The German advisory committee has advised that activities with gene drives should be classified at containment level II as a minimum. In this way, the risk assessment was also brought to the attention of the German government.

The regulations on governmental review of the risk assessment differ between EU member states. For example, the classification at

containment level IV and assessment through an Article 2.8 procedure is a unique procedure that is only required in the Netherlands. The Netherlands is the only EU member state that has implemented a specific statutory regulation for gene drive technology.

The risk assessment of gene drive technology differs from regular genetic modifications; it is not the pathogenicity that has to be assessed, but the risk that an organism with a gene drive will spread from the laboratory to the surrounding environment. The experts thus confirmed the earlier conclusion of RIVM that pathogenicity in gene drive technology is not the only relevant aspect for the risk assessment and that it is not yet sufficiently known how harmful effects due to the spread of the organism can and should be assessed.

The experts also concluded that harmful effects other than pathogenicity were formulated in the Directive and that the risk assessment method according to this Directive provides an effective starting point for assessing gene drive technology.

During the working session, risk assessments were discussed for using gene drives in three model organisms: yeast, mice and fruit flies. From the plenary discussion it emerged that there was no need to develop risk assessments for individual groups of organisms because the same questions always turned out to be relevant: what is the biology that the organism uses to spread itself and what is the genetic composition of the gene drive? The three organisms mentioned above are interesting for applications of gene drive technology not only because they are used as a model organisms in research, but also because future releases into the environment of rodents or arthropods with a gene drive can be expected (e.g. mosquitos that can no longer transmit malaria parasites).

4 Specification of the risk assessment method and risk management measures

4.1 Specification of the risk assessment method

4.1.1 *Research question and approach*

A risk assessment must be conducted to establish measures to ensure that the risk of a GMO for humans and the environment is negligible. This is done by defining the potentially harmful effects of the GMO in advance. Subsequently, based on information about the severity of the effects and the likelihood that they can occur, the risk that these effects could occur as a result of the unintentional release of the GMO from the laboratory into the environment is determined.

The outcome of a risk assessment is a risk class on the basis of which containment measures can be established that lead to a negligible risk of unintentional release of the GMO from the laboratory.

Research question 1:

How can the risk assessment method for contained use of GMOs with a gene drive be specified so that adequate risk management measures can be established?

For the specification of the risk assessment method for organisms with a gene drive, the working session ascertained that the method prescribed in the Directive offers effective starting points. This method is shown schematically in Appendix 1 of this report, and consists of three elements: 1) identification of the potentially harmful effects, 2) determination of the severity and likelihood that these effects can occur and 3) determination of the risk class. The specification of this method for gene drive technology has been investigated by the working group and is described below.

4.1.2 *Specification of the risk assessment method*

4.1.2.1 *Identification of potentially harmful effects*

The potentially harmful effects of a GMO that are listed in the Directive are shown in the text box below.

In the case of gene drive technology, this is an organism that is genetically modified with a gene drive (i.e. with a sequence-specific endonuclease, see 1.2). Instead of 'GMO', in this text the term 'gene drive organism' (GDO) is used to indicate that this specific group of GMOs is involved.

Potentially harmful effects of a GMO as formulated in 2009/41/EC [7]:

The following potentially harmful effects should be considered:

- disease to humans, including allergenic or toxic effects;
- disease to animals or plants;
- deleterious effects due to the impossibility of treating a disease or providing an effective prophylaxis,
- deleterious effects due to *establishment or dissemination in the*

- environment,*
- deleterious effects due to the natural *transfer of inserted genetic material to other organisms.*

The underlined text indicates the harmful effects that are specific to a GDO. These harmful effects can be expressed if the GDO is released into the environment as a result of an incident and can cause the genetic trait (the gene drive) to spread in a natural population through establishment or spread in the environment.

If a GDO is a pathogenic organism or if the gene drive alters the disease-causing potential of the organism, then harmful effects that relate to these aspects must also be included in the risk assessment. In this report, however, we have not considered the assessment of such effects because ample experience has already been acquired in this area with GMOs.

4.1.2.2 *Determination of severity and likelihood*

After determining the potential harmful effects of a GDO, the severity of these effects is determined on the basis of the characteristics of (1) the organism and (2) the specific modification. This is done by gathering information about the biological properties of the organism that relate to its method of spreading. The information that is required about the modification concerns the genetic composition of the gene drive and the possible location(s) of the inserted genetic material in the genome. Based on this information, the severity of the potentially harmful effects of the resulting GDO is determined.

Besides determining the severity, the likelihood that a harmful effect can occur is also determined. This likelihood is determined by assessing the specific activities in the laboratory, such as the culturing/breeding conditions and the scale of the activities. In case of arthropods, for example, the scale would refer to the numbers of arthropods and the group size. Regarding specific laboratory activities, for instance, opening a breeding tray with non-anesthetised flying arthropods results in a greater chance of escape than when the arthropods are anesthetised before the tray is opened.

The questions about the severity and likelihood that harmful effects can occur as a result of an activity with a GDO are part of the current risk assessment for GMOs (see Annex III of the Directive and Appendix 8 of the GMO Regulation).

4.1.2.3 *Determination of the GDO risk class*

The final step in the risk assessment is to determine the risk class for the relevant activity with the GDO.

A risk class allows grouping of activities of GDOs with a similar level of risk and for which a comparable set of containment measures is sufficient.

The risk classes proposed in the Directive concern four classes based on pathogenicity. However, this classification cannot be used for GDOs. The working group therefore proposed the following risk classes for activities with GDOs:

GDO risk class 1²: the activities with a GDO lead to a negligible or low risk for humans and the environment. A GDO that belongs to this class is essentially a GMO whose gene drive cannot spread, such as a GDO that cannot survive in the environment outside the laboratory.

GDO risk class 2: the activities with a GDO have a medium risk. This would apply to an activity with a GDO that can cause short-term harmful effects on humans and the environment, but not permanent ones, or if the original situation can be restored by, for example, spraying the surrounding environment with insecticides. In the case of a short-term risk, for example, a 'daisy gene drive system' [9] can be considered, whereby only a few generations of offspring will inherit the gene drive.

GDO risk class 3: the activities with a GDO have a high risk. In that case there is a possibility that the GDO can cause a permanent harmful effect by becoming established in the environment and/or by spreading the gene drive to offspring.

If the GDO is pathogenic, then the harmful effects due to the pathogenicity must also be taken into account. In that case, besides the GDO risk classes, the risk classes for pathogenicity must also be included when determining the outcome of the risk assessment. The subsequent risk management measures will then consist of a combination of measures that emerge from both risk classes.

4.2 Determining the risk management

4.2.1 *Research question and approach*

The outcome of the risk assessment is a GDO risk class that applies to the assessed activity with the GDO. Risk management is based on the GDO risk class (or multiple risk classes if pathogenicity is also involved): this is the set of containment measures that is necessary to ensure that the activity with the GDO has a negligible risk. The second research question concerns the determination of the specific containment measures that are needed to ensure a negligible risk.

Research question 2:

What containment measures are needed to ensure that the risk of a GDO spreading into the environment is negligible?

The Directive specifies risk classes for activities with pathogenic microorganisms and sets of measures that are tailored to the harmful effects of these organisms. These measures are less suitable for organisms that are not pathogenic or are not microorganisms. Consequently, research must be conducted into what other measures are possible and necessary to ensure that the risk of GDOs spreading is negligible.

² When the term 'risk class' is used in the report, this refers to the risk class of a GMO as indicated in the Directive and the GMO Regulation. When this report refers to the risk class of a GDO, this indicates an explicit GDO risk class.

4.2.2 *Proposal for containment measures*

A GDO can in principle be any sexually reproducing organism. The results from the working session and the review of scientific literature show that fungi (including yeast), arthropods and rodents will be the most likely organisms to be genetically modified with a gene drive. Much is known about their biological characteristics and spreading potential of these groups of organisms. Moreover, these organisms are frequently included in GMO risk assessments for contained use. Because the biology of these groups of organisms differs greatly, the proposed containment measures also distinguish between measures for yeast, arthropods and rodents.

By definition, activities with a GDO in risk class 1 have a risk that is comparable to activities with a GMO in risk class 1; for organisms in this category, no harmful effects resulting from its increased spread or its genetic trait (the gene drive) have been ascertained. For GDOs in risk class 1, therefore, containment level 1 will be sufficient for activities with fungi and animals in accordance with the containment measures specified in Table IA (laboratory activities) and Table IC (activities in animal units) of the Directive. In Dutch legislation, these containment measures are specified in Appendix 9 of the Regulation, under 9.1.1.1 (ML-I) for fungi and 9.1.4.1 (D-I) for animals.

When determining the containment measures for activities with GDOs in risk classes 2 and 3, the specific biology of the organism is taken into account to prevent its escape from the laboratory. The proposed measures are based on the expertise of the working group and the measures described in the Directive and the guidance on containment measures for arthropods [10].

Analysis of these measures has resulted in sets of minimum containment measures that are required for the various groups of organisms and the various GDO risk classes. These measures are summarised in the table in Appendix 2.

The measures for activities with GDOs in risk classes 2 and 3 involve, among other things, additional physical barriers between the organism and the environment, stricter rules for access to the laboratory and a plan to detect the escape of a GDO from its cage. For activities with GDOs in risk class 3, a mandatory emergency plan has been proposed that takes effect in the event of an incident.

After determining the necessary containment measures based on the GDO risk class of the relevant activity, the risk assessment is repeated (iterative process). This iterative process is used to determine whether the measures are indeed sufficient to ensure a negligible risk of the activity with the GDO. If this is not the case, the measures must be revised. In case of an activity with a pathogenic GDO, the iterative process is especially important. To ensure that the risk of all harmful effects is negligible, two sets of measures are required: those associated with the risk class of the GDO, and those associated the risk class of the pathogen.

In Appendix 3, two examples of a risk assessment have been elaborated on the basis of published activities with a GDO.

4.3 Incident management

4.3.1 *Research question and approach*

In the event that a GMO escapes from the laboratory and is released into the environment, procedures must be in place that specify the required actions and measures (see Article 13 of the Directive and Article 9, second paragraph of the GMO Regulation). Although incident management is not a statutory task for the authorising body, RIVM was requested to investigate this aspect. The working group therefore conducted a brief survey of the possibilities for incident management based on the relevant literature and their own expertise (see 4.3.2).

Research question 3:

What are the possibilities for incident management?

4.3.2 *Possibilities for incident management*

Various measures are conceivable that can limit a harmful effect resulting from the unintentional release of a GDO from the laboratory. For instance, if the GDO is an arthropod, an additional introduction into the environment of the wild-type organism can limit the spread of the GDO in certain cases. An example of the above could be the introduction of a wild-type organism that is insensitive to the sequence-specific endonuclease due to the genetic variation in the DNA sequence of the cleavage site. Another possible incident management measure is to kill the GDO with an insecticide.

The working practices (see Appendix 2) for activities with rodents require that individual animals are clearly identified as GDOs so they can be traced after an incident has taken place.

Esvelt *et al.* [11] described the possibility of developing a reverse gene drive: a second GDO that can restore the natural phenotype. However, this method is not an obvious choice following an incident. This is because the introduction of a GMO into the environment requires authorisation under Directive 2001/18/EC [12]. Such an authorisation would have to be requested simultaneously with the authorisation for the initial contained use activity. More important, however, is that there must be clarity in advance about the effectiveness of this reverse gene drive.

If the risk assessment of an activity with a GDO from risk class 3 indicates that the risk will not be negligible with the proposed containment measures, it can be decided to deny authorisation of the activity. In that case, a possible solution can be found by first gathering more information about the spreading potential of the GDO based on the wild-type organism and the natural population. Another option is to perform activities with a lower-risk GDO by using ecological or biological containment measures (e.g. a strain that cannot survive outside the laboratory or the use of an insertion site of the gene drive in the genome that is unique to a laboratory strain).

During its research, the working group noted that transport is an activity during which escape is very conceivable. Although transport is regulated by other legislation, it may be desirable to point out that a GDO from a risk class 3 activity should not be transported; transport is permitted only if the wild-type organism and the DNA construct are shipped separately.

5 Conclusions and recommendations

5.1 Conclusions

The present study focussed on specifying a risk assessment method for organisms with a gene drive, determining the corresponding containment measures and identifying the possibilities for incident management. This study has led to the following conclusions:

Risk assessment method

- Extensive experience has been gained with the method specified in the Directive for assessing GMOs under contained use. However, the potential harmful effects of a GDO are different from those of most GMOs to which this experience applies. During the working session it was concluded that the general principles of the method appear to be applicable to the risk assessment of a GDO.
- The results of the working group show that the potential harmful effects of a GDO are also covered by this method. Moreover, the potential harmful effects can be assessed with the questions that are already defined to gather information about the likelihood that a GMO can cause these effects and about their severity.
- For organisms with a gene drive, three new GDO risk classes are proposed as outcomes of the risk assessment that are classified according to the degree of risk of spreading the GDO and/or the genetic trait.

Risk Management

- For each group of organisms (fungi, arthropods and rodents), minimum sets of containment measures are proposed for each GDO risk class.
- For activities with a GDO from risk class 1, the risk is comparable to an activity with a GMO in risk class 1 or containment level 1, and similar containment measures can be used.
- For activities with a GDO from risk classes 2 and 3, taking into account the biology of the organism, specific measures have been drawn up to contain the organism within the laboratory. The table in Appendix 2 provides an overview of sets of minimum containment measures that are required for the various groups of organisms and various GDO risk classes.
- However, it may be necessary to deny authorisation if the outcome of the risk assessment and the proposed risk management measures do not result in a negligible risk.

Incident Management

- To respond adequately in the event of incidents, rules for GDO risk classes 2 and 3 have been proposed, such as identification of the GDO, to ensure that tracing is possible. Furthermore, various forms of incident management have been proposed.

Knowledge and information

- As a result of the knowledge and information gained through interviews conducted with research groups in the Netherlands and the meeting with international experts, the working group began specifying a risk assessment method based on the European Directive 2009/41/EC [7] and developing risk management for three groups of organisms.
- The previously identified knowledge gaps that RIVM reported in 2015 [1] have been filled with regard to the adequate completion of the risk assessment method and a proposal for suitable containment measures. To develop further knowledge, experience with using this method is still required.
- Scientific research continues to provide new knowledge and information about gene drive technology. It is crucial to keep track of this research so new information can be used in the risk assessment and new applications of the technology can be adequately assessed.

5.2 Recommendations

5.2.1

Knowledge development and information acquisition

Researchers sounded the alarm about gene drive technology in 2014 [13], even before the technology was used, but worried about the high risks that could be involved. If insufficient data are available on the exact composition and functioning of a specific GDO, the risk assessment must take a high risk of spreading (*worst case scenario*) into account. Such a GDO will therefore fall into GDO risk class 3.

In recent years, much research on gene drive technology has been initiated. Fundamental knowledge is being acquired under contained use and the risk potential can change during the development of the corresponding technology. This has generated the following insights:

- Gene drives can be constructed in various ways. With two new approaches, the potential risk due to spreading of a GDO outside the laboratory has become medium to negligible (e.g. daisy gene drive [9], split gene drive [14]).
- Resistance can occur. In this case, the cellular repair mechanism makes errors in the DNA sequence of the cleavage site. As a result it is no longer recognised by the sequence-specific endonuclease of the gene drive. The gene drive can therefore no longer be copied and transferred to offspring. The spreading of the gene drive into the population therefore stops [15].
- Genetic variation at the cleavage site for the sequence-specific endonuclease can prevent spreading. Due to this genetic variation, not all individuals in a population will be able to copy the gene drive and pass it on to nearly all their offspring. Therefore, the gene drive cannot spread within an entire population [16].

Based on more information about the potential of the gene drive to spread in a population, the risk assessment can lead to a more precise determination of a GDO risk class and to additional and/or substitute containment measures that may be required for certain activities. Instead of taking a *worst case scenario* as a starting point, on the basis

of research results it can be determined that a lower GDO risk class, possibly with additional/substitute containment measures, is appropriate.

Recommendation:

Continuing to track developments in gene drive technology by maintaining connections with international networks is crucial because it contributes to the knowledge about the functioning of gene drives and the ability to perform an adequate risk assessment.

5.2.2 *International discussion*

The findings from the RIVM working session (2.3) and working group (2.4) with regard to the risk assessment of a GDO activity make it clear that it is desirable to reach European-wide agreement on a uniform risk assessment and risk management. Indeed, if an organism with a gene drive is placed in a risk class that is too low and is unintentionally released from a laboratory, then it may become established in the environment and spread across an international border. In this scenario, the experts concluded that neighbouring countries had to be informed when an incident occurred with a potentially severe risk to humans and the environment. There are provisions for this in the Directive (e.g. Article 13). At present it is unclear how other EU member states deal with the assessment of GDOs and whether they report incidents with GDOs as severe and subject to Article 13.

Recommendation:

Conducting a European discussion on a harmonised procedure for the risk assessment of activities with GDOs under contained use is urgent to prevent these activities from being conducted with inadequate containment measures. Agreements are also needed about the way in which information must be exchanged if an incident with a GDO takes place that can have severe consequences for humans and the environment.

6 Recommendations tailored to the authorisation system in the Netherlands

6.1 Request for authorisation

Article 5.0 in Appendix 5 of the GMO Regulation (see also 1.2) applies specifically to the classification of an activity with a GDO. This article refers to level IV for an activity with a GDO and therefore requires authorisation. To determine the appropriate containment measures, a request can be submitted on the basis of an Article 2.8 procedure. The 2.8 procedure is frequently used for various GMO activities that cannot be classified according to Appendix 5. This is also a workable procedure for a GDO activity.

To date, no requests for authorisation concerning activities with GDOs have been submitted. Once a number of requests have been submitted and experience has been gained with the classification into GDO risk classes, it will become possible to amend the classification article. Such an amendment could involve the specification of the classification article based on the three GDO risk classes. In that case, the specification must correspond with the existing or new classes of physical containment (CFIs in Dutch) in Appendix 9 of the GMO Regulation (see 6.3).

Recommendation:

Article 5.0 (on classification) can continue to apply; it facilitates a procedure for requesting authorisation for activities with GDOs on the basis of article 2.8 of the GMO Decree [5]. Once a number of requests have been submitted and experience has been gained with the classification into GDO risk classes, the redefinition of Article 5 to link up with these GDO risk classes can be considered.

6.2 Risk assessment of organisms with a gene drive according to the method in Appendix 8 of the GMO Regulation

The results from Chapter 4 describe how a risk assessment for GDOs can be implemented. This risk assessment method is based on Annex III of the Directive and is also laid down in Appendix 8 of the GMO Regulation. If a user submits a request based on Article 2.8, a risk assessment must be carried out as indicated in Appendix 8, in which the outcome of the risk assessment refers to containment levels instead of risk classes. In addition, Appendix 8 provides rules of thumb that help to determine the correct containment level and the corresponding CFI. A CFI consists of a set of containment measures intended for an activity with a GMO in a laboratory, greenhouse, animal enclosure or process installation. These sets of containment measures are specified in Appendix 9 of the GMO Regulation.

However, Appendix 8 does not provide a rule of thumb to arrive at a risk class (or containment level) for an activity with a GDO. Because no experience has yet been gained with the use of GDO risk classes, it is too early to amend the appendix to be compatible with these classes. Nevertheless, applicants for authorisation should be informed about the

implementation of the GDO risk classes as proposed in this report. For new developments in the field of genetic modification, it is customary to provide information regarding the safety of these developments via the website <https://ggo-vergunningverlening.nl> or to share this information with the applicant if asked.

Recommendation:

Appendix 8 helps the user to perform an adequate risk assessment for GDOs, but users need additional guidance on how to apply the three GDO risk classes.

6.3 Determination of containment measures according to Appendix 9

Based on the containment level and the associated CFI that emerge from the risk assessment, the applicant can determine the corresponding containment measures by referring to Appendix 9. Appendix 9 lists the mandatory containment measures for the CFIs corresponding with activities with GMOs in laboratories, greenhouses, animal housing and process installations.

However, the GDO risk classes and the corresponding sets of minimum containment measures for GDOs described in this report are not entirely consistent with the CFI categories ML-I, ML-II, ML-III, D-I, DM-II and DM-III. For example, measures for DM-II and DM-III have been prescribed that relate to the containment of microorganisms, but these are not relevant to an arthropod or rodent with a gene drive. To tailor the containment measures to activities with a GDO, the GMO Regulation makes it possible to impose additional requirements on an applicant for specific activities by means of Article 2.21 (working practices) and Articles 2.56 and 2.57 (facility measures). These requirements are in addition to the current measures of the CFIs. These articles also offer the possibility to impose alternative measures to replace the standard requirements of the CFIs.

Because the required containment measures can be individualised, there is currently no need to establish new CFIs for organisms with a gene drive due to their increased spreading potential. Furthermore, no requests for authorisation have yet been made for activities with gene drive organisms in the Netherlands, and no definitive sets of containment measures have been determined. An additional consideration is that this would involve the inclusion of 6 new CFIs in Appendix 9, in any case for 3 groups of organisms (fungi, rodents, arthropods) in risk classes 2 and 3. It should be noted that the CFIs for risk class 1 will be equivalent to ML-I and D-I.

Recommendation:

Current regulations make it possible to individualise the specification of mandatory containment measures. It is therefore not necessary to supplement Appendix 9 with new CFIs and/or sets of additional and substitute measures.

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Appendix 1 Risk assessment method for GMOs in accordance with Directive 2009/41/EC

The schematic in Figure 1 shows the steps of the risk assessment method for genetically modified organisms (GMOs). The risk assessment begins with the determination of the potentially harmful effects that may occur as a result of the escape of the GMO from containment. This concerns the potentially harmful effects for humans, animals, plants or the environment.

The severity of a harmful effect is determined on the basis of the characteristics of the organism and the genetic modification. The likelihood that a harmful effect can occur is determined by the specific activities in the laboratory and the environment outside the laboratory. Both steps (the determination of severity and likelihood) determine which risk class should be assigned to the activity with the specific GMO.

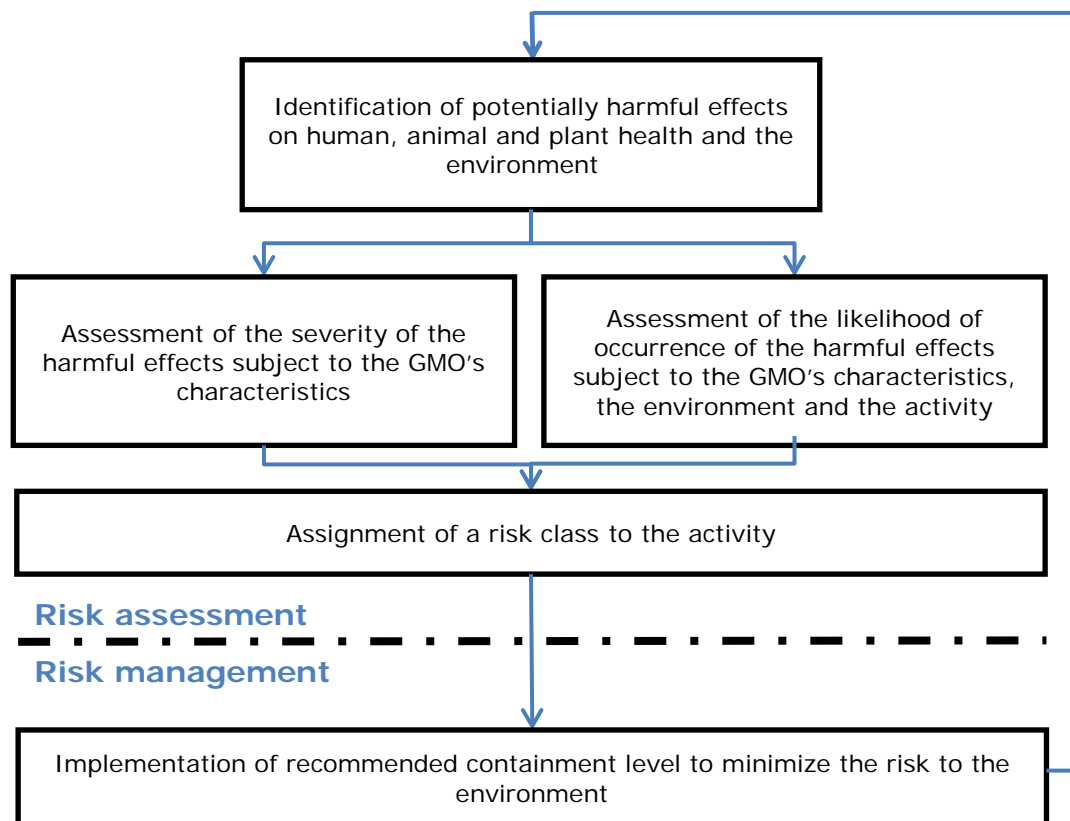


Figure 1: Schematic representation of the risk assessment method for activities with GMOs under contained use.

Based on the risk class, the containment level (the category of physical containment – abbreviated as CFI in Dutch – in the GMO Regulation) is established. Subsequently, the risk assessment is repeated to determine whether the containment measures are indeed sufficient to ensure that the risk of the activity with the GMO is negligible. If this is not the case,

the containment measures will be adapted. Therefore, determining the correct containment measures takes place through an iterative process.

Appendix 2 Table of minimum containment measures required for a contained use activity with a GDO from risk class 1, 2 or 3

	Minimum containment measures		
	GDO risk class 1	GDO risk class 2	GDO risk class 3
Physical measures	Two layers of physical containment: (1) a species-appropriate container (unbreakable, escape-proof) and (2) a laboratory to include species-specific barriers	Additional third layer of physical containment o enclose the species appropriate container	
			2-door system with interlock
Working instructions		Access to all areas used for GDO activities limited to trained personnel and instructed service personnel	Access to all areas used for GDO activities restricted to trained personnel and accompanied service personnel
		Monitoring plan available to test for the presence of the gene drive element(s) in the environment in case of unintentional release	
			Emergency plan is available in case of detection of gene drive elements in the environment
Additional containment measures – species-specific			
For activities with yeast and filamentous fungi		All manipulations inside a Class II biosafety cabinet	
			Airlock, laboratory with negative pressure relative to surroundings, and HEPA-filtered exhaust The controlled area must be sealable to permit fumigation

For activities with arthropods	Hanging curtain at laboratory side of door	Two-door system with interlock	
	Arthropods are immobilised for handling		Arthropods immobilized for handling and handled inside closed containment (e.g. inside a tent)
			Program to monitor the effectiveness of escape prevention
			Protocols are practiced with wild-type organisms before implementation
		All manipulations with GDOs to be observed by second trained individual to provide assistance and verify adherence to procedures	
For activities with rodents			Identification of animals (earmark, chip, etc.) is recommended
			Means to identify animals (earmark, chip, etc.)
		Camera or window to monitor housing of rodents	

Appendix 3 Detailed examples of the risk assessment of activities with a GDO

The following examples concern the risk assessment of activities with GDOs as described in the scientific literature, according to the method described in Chapter 4 of this report.

Example 1

Characteristics of the GDO to be assessed: The mosquito *A. gambiae* is the recipient organism for a molecular construct carrying a gRNA and Cas9 encoding sequences flanked by homologous sequences to a resident female fertility gene that confers a recessive female-sterility phenotype upon disruption [17]. Manipulations involve feeding and rearing GDOs including transfer to cages and microscopy with live mosquitoes in a laboratory in London. Currently, members of the *A. gambiae* complex are found throughout tropical Africa but are not endemic in Europe or Northern America [18].

Risk assessment: This GDO is able to drive the female fertility gene to 91,4 to 99,6% of its progeny [17] and has a high risk of suppressing each permissive mosquito population. The **severity** that potential harmful effects may occur is **high**.

Considering the type of activity (open phases) and the type of organisms (flying ability, small size and hiding capabilities) the likelihood that a harmful effect may occur is high. However, due to the location of the laboratory in the Northern temperate climate, this mosquito species is ecologically contained and would not survive nor transfer the gene drive construct to offspring if unintentionally released in the local environment. The **likelihood** is thus **negligible**.

Combining the high severity and negligible likelihood of occurrence, and taking into account the definitions of the GDO risk classes, GDO risk class 1 is assigned to these activities.

Risk management: The assignment of GDO risk class 1 to the activity enables the user to select the minimal containment measures from Appendix 2. The measures to be implemented in order to prevent the potential harmful effects (i.e. survival in the environment or transfer of the gene drive to wild relatives) are similar to those measures to be taken for a conventional genetically modified arthropod.

It is clear that if the ecological containment was not in place, this activity would be assigned to risk class 3 due to the high severity of the potential harmful effects.

Example 2

Characteristics of the GDO to be assessed: Wild mice (*M. musculus*) are the recipient organism for a daisy drive construct. Each generation of mating with wild-type organisms will reduce the average number of guide RNA elements per GDO by half, serving as a generational clock [19].

Manipulations involve feeding and rearing GDOs including transfer to cages and collecting blood samples.

Mice have a natural habitat outside of the laboratory environment and may reproduce with relatives in the environment.

Risk assessment: To assess the severity of harmful effects due to this activity the molecular design of the gene drive construct as a daisy drive is considered. In this case, the percentage of offspring that will inherit the gene drive construct decreases over a few generations. An unintentional release may thus result in a few generations carrying a gene drive, but long-term establishment in the environment is not anticipated. The **severity** is therefore classified as **medium**.

The **likelihood** that a harmful effect may occur is **medium** as the unintentional release of a mouse from the laboratory during manipulations is possible but only moderately likely. Mice are quite visible and easy to catch and manipulations are generally performed on a low number of animals at any given time.

Combining the medium severity and medium likelihood of occurrence, and taking into account the definitions of the GDO risk classes, GDO risk class 2 is assigned to these activities.

Risk management: In accordance with Appendix 2 an additional layer of physical containment, restricted access, a monitoring plan and identification of animals is recommended for a GDO risk class 2 activity. These measures should prevent or enable the detection of an unintentional release while still being proportionate to a non-permanent establishment of the GDO in the environment.

