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

Assessment of human health and environmental risks of new developments in modern biotechnology

Policy report

RIVM Letter report 2018-0089
P.A.M. Hogervorst et al.

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Colophon

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Synopsis

Assessment of human health and environmental risks of new developments in modern biotechnology
Policy report

Due to the rapid developments in modern biotechnology, many new applications are expected in the next ten years. To be prepared, RIVM has investigated whether the current risk assessment for human health and the environment is still adequate. This was done for a selection of nearly thirty new applications. The current risk assessment appears to be adequate for about half of these. For the other half, the risk assessment method may no longer be adequate, or insufficient knowledge or information is available to effectively assess risks.

In the present study the risk assessment method for genetically modified organisms was reviewed. This method is used for living organisms whose genetic material has been modified, as has been the case for most current biotechnology applications. However, some new applications do not consist of living organisms. In the near future, for example, this will be the case for RNA sprays, which are used to suppress pests on crops. For such applications, the current risk assessment method may not be the best choice. For some applications that are still at an early stage of development, it remains unclear whether the current assessment method is usable. This applies, for example, to 'orthogonal systems', which use biochemical building blocks or DNA coding systems that are not found in nature.

To deal with the expected bottlenecks in the current risk assessment, there is a need to draw lessons from other risk assessment methods, to gather existing information and knowledge and to fill knowledge gaps.

Keywords: biotechnology, new developments, risk assessment, genetically modified organisms, genome editing, regulation of gene expression, synthetic biology, safety, human health, environment

Publiekssamenvatting

Beoordeling van risico's voor mens en milieu van nieuwe ontwikkelingen in de moderne biotechnologie

Beleidssignalering

Door de snelle ontwikkelingen in de moderne biotechnologie, worden er in de komende tien jaar veel nieuwe toepassingen verwacht. Om hierop voorbereid te zijn heeft het RIVM onderzocht of de huidige risicobeoordeling voor mens en milieu nog volstaat. Dit is gedaan voor bijna dertig geselecteerde nieuwe toepassingen. De huidige risicobeoordeling blijkt voor de helft van deze toepassingen op orde te zijn. Voor de andere helft van de onderzochte toepassingen zal de methode van risicobeoordeling (mogelijk) niet meer passen of is er onvoldoende kennis of informatie om de risico's voor mens en milieu goed te kunnen beoordelen.

In dit onderzoek is de risicobeoordelingsmethode voor genetisch gemodificeerde organismen getoetst. Deze methode is opgezet voor levende organismen waarvan het erfelijk materiaal is aangepast, zoals tot nu toe bij de meeste biotechnologische toepassingen het geval is. Er komen nu ook toepassingen aan die niet bestaan uit organismen, en waarvoor deze risicobeoordelingsmethode dus niet logischerwijs het meest geëigend is. Op de korte termijn geldt dat bijvoorbeeld voor de zogeheten RNA-spray, waarmee plaaginsecten op gewassen worden onderdrukt. Voor enkele toepassingen die nog in een vroeg ontwikkelingsstadium zijn, is nu nog onduidelijk of de bestaande beoordelingsmethode bruikbaar is. Dit geldt bijvoorbeeld voor 'orthogonale systemen' waarbij andere bouwstenen of een andere codering van DNA wordt gebruikt dan nu in de natuur voorkomt.

Om de verwachte knelpunten in de risicobeoordeling op te lossen, is het nodig om lering te trekken uit andere bestaande risicobeoordelingsmethoden, bestaande informatie en kennis bij elkaar te brengen en om ontbrekende kennis op te bouwen.

Kernwoorden: biotechnologie, nieuwe ontwikkelingen, risicobeoordeling, genetisch gemodificeerde organismen, genome editing, regulatie genexpressie, synthetische biologie, veiligheid, mens, milieu

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Summary

In biotechnology, far-reaching developments are occurring at a rapid pace, with applications in sectors such as agriculture, medicine and industry. These developments are based on breakthroughs in modification of DNA, the regulation of gene expression and synthetic biology. Many applications of these developments promise to contribute to solving societal problems such as hereditary diseases, environmentally harmful industry or unsustainable agriculture. On the other hand, there is uncertainty about the risks for human health and the environment from new biotechnological applications.

Recently, various national and international forums[1] have noted that the new developments in modern biotechnology are leading to new questions for risk assessment. Against this background, the Ministry of Infrastructure and Water Management has commissioned RIVM to investigate whether new developments in modern biotechnology can be assessed for risks to human health and the environment using the current risk assessment method for genetically modified organisms (GMOs).

To address these questions, it is necessary to look into the applications of these new developments in more detail. For this reason, 28 biotechnological applications were selected and studied, the majority of which are expected within the next ten years. We concluded that:

- The risks of half of the 28 studied applications can be assessed with the existing method.
- For a small proportion of the applications, the existing method of risk assessment is unsuitable, or it is still uncertain whether this method is suitable for their assessment.
- For a few applications, additional questions may arise about the most suitable method of risk assessment; this is because these applications do not involve modification of the genetic material of living organisms.
- For the risk assessment of about one-third of the applications, more knowledge and/or more information is needed to conduct an adequate risk assessment.

This summary is structured as follows:

1. a description of the research approach;
2. a synopsis of the applications studied and a presentation of the research results, ranked according to urgency and complexity of the risk assessment;
3. the conclusions that were drawn; and
4. a discussion that places the research in a broader perspective.

[1] For example, the Netherlands Commission on Genetic Modification (COGEM), the European Commission's scientific committees (the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Consumer Safety (SCCS)), the Convention on Biological Diversity of the United Nations (CBD) and the National Academy of Sciences of the United States (NAS) have warned that contemporary developments in biotechnology have raised questions about risk assessment.

Box 1: Illustration of biotechnological applications that are expected in various sectors, their potential significance for solving societal challenges, and the risks they entail.

Examples of new applications of biotechnology in various sectors

In the medical sector new forms of gene therapy are emerging, sometimes with the possibility to repair or remove sequences in the germline. On the one hand, this helps to control hereditary diseases, but on the other hand, there are questions about the safety of such interventions.

In the industrial sector there are applications of genetically modified algae to produce precursors of products such as plastic, oil or ethanol. GMOs like these give an impetus to the biobased economy, but also raise questions about how algae behave as hosts, the environmental consequences of a release, survival of genetically modified algae, and which containment measures can be taken.

In agriculture, plants can be genetically modified to influence the microbiome on and around their roots. This can enhance nitrogen fixation and help prevent disease. However, due to the limited knowledge about the microbiome of plants and the complex interaction with the soil ecosystem, the consequences for the soil ecosystem functions are more difficult to assess.

The use of some applications is not limited to a single sector. For example, gene drives make it possible to reduce or genetically adapt entire populations of sexually reproducing organisms. This has potential applications such as the control of infectious diseases, of agricultural pests or the prevention or restoration of ecological damage by invasive species. While the advantages of this technique are clear, there is uncertainty about potential adverse effects, such as the inadvertent reduction of entire populations of beneficial organisms. This requires, more than for plants, assessment of effects at the population level.

1. Approach of the study

Delineation of the study

The underlying study focused on the method of risk assessment, but separate from the existing regulatory frameworks. The study did not attempt to answer the question of whether the legal/regulatory frameworks that now require a risk assessment for biotechnological applications are also appropriate for future applications. Nor did this study focus on the ways in which current developments in biotechnology are interwoven with ethical, socio-economic or biosecurity issues (securing biotechnological applications and knowledge to prevent misuse).

Method

The study was conducted in five steps:

1. A selection was made of biotechnological applications to analyse whether the existing risk assessment method is sufficient to assess the risks of these applications. Box 1 lists the inclusion criteria for the applications.

2. For each of the selected applications it was determined, based on expert judgment, whether possible risks can be adequately assessed with the existing risk assessment method. During this process the various experts always used the same methodology.
3. The results of Step 2 were submitted for review by internal and external experts in risk assessment of biotechnology and other fields.
4. Based on the feedback from Step 3, the methodology was revised and the suitability of the existing risk assessment method for each of the selected applications was tested again. Figure 1 shows the revised methodology.
5. Based on the analysis of the outcomes of the 28 individual tests, conclusions were drawn at both the individual application level and the aggregate level about the continued applicability of the current risk assessment method for biotechnological applications. This is summarised in Figures 2, 3 and 4 and Table 1.

Box 2: Inclusion criteria used in selecting biotechnological applications to analyse the existing risk assessment method with the aim of determining its continued applicability.

Inclusion criteria for the applications

a) Individual applications were classified according to three time periods in which they will probably be introduced:
   - 0-5 years,
   - 5-10 years, or
   - more than 10 years;

   The collection as a whole contains:

b) applications that are
   - used only under containment, and
   - those that are deliberately introduced into the environment;

c) applications in which the underlying technique is
   - modification of DNA,
   - regulation of gene expression, and
   - synthetic biology;

d) applications that can be used in
   - the medical sector,
   - the industrial sector,
   - the agricultural sector, or
   - other sectors.

Finally, we selected the applications in such a way that the complete set would contain examples of all possible combinations of values listed under criteria b, c and d, and that as many of the selected applications as possible would be expected within ten years.

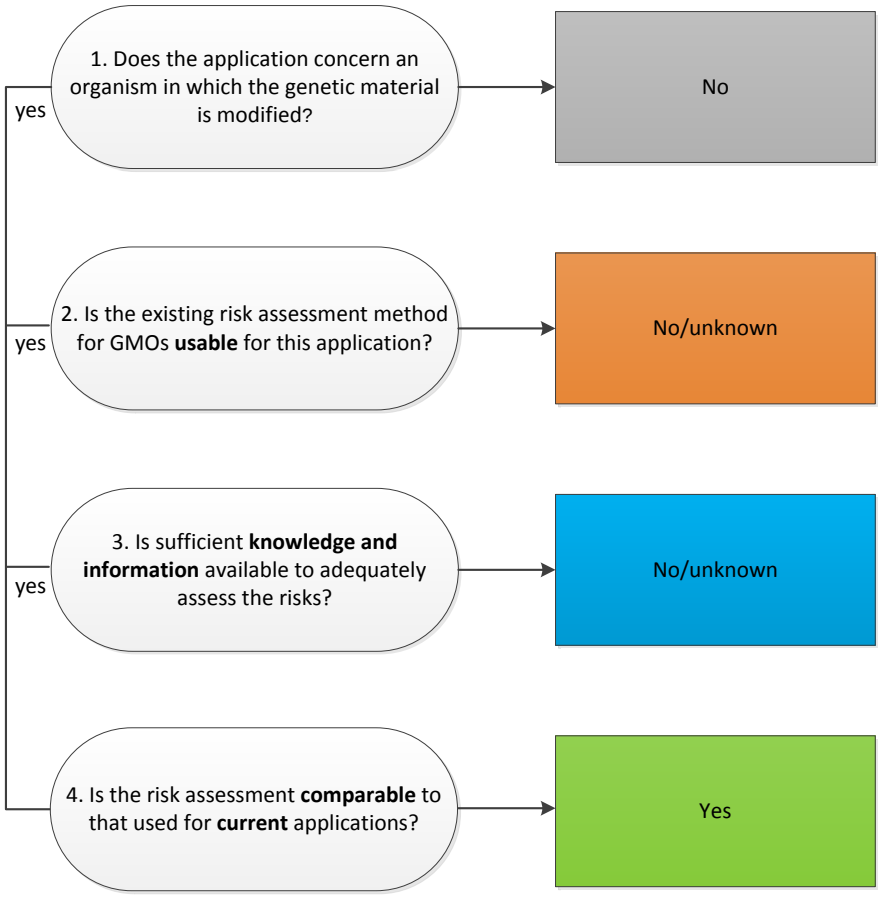

Figure 1: Schematic representation of the question structure that was used when determining whether an adequate assessment of risks for human health and the environment of new biotechnological applications can be performed.

By going through the question structure for each of the 28 selected applications, it was determined for each application whether a risk assessment can be performed with the existing assessment method. If Question 1 can be answered with 'yes', the existing risk assessment method for GMOs is expected to be suitable. If the answer is 'no', then the risk assessment for GMOs is not suitable for this application. Question 2 distinguishes between applications for which the existing risk assessment method for GMOs is suitable and applications for which this is not the case, or for which the suitability is uncertain. Question 3 helps to establish whether sufficient information is available to adequately assess the potential risks for human health and the environment and if additional knowledge is required to actually perform this assessment. Question 4 is a control question that helps to determine the definitive selection of applications for which the existing risk assessment method is adequate.

2. Results

The selection process yielded 28 applications. These are shown in Table 1. Here the applications are classified according to whether they are used under containment or are introduced into the environment, examples are given, and the result of the confrontation of each

application with the methodology as shown in Figure 1 are indicated with a colour. A schematic summary is shown below in Figure 2.

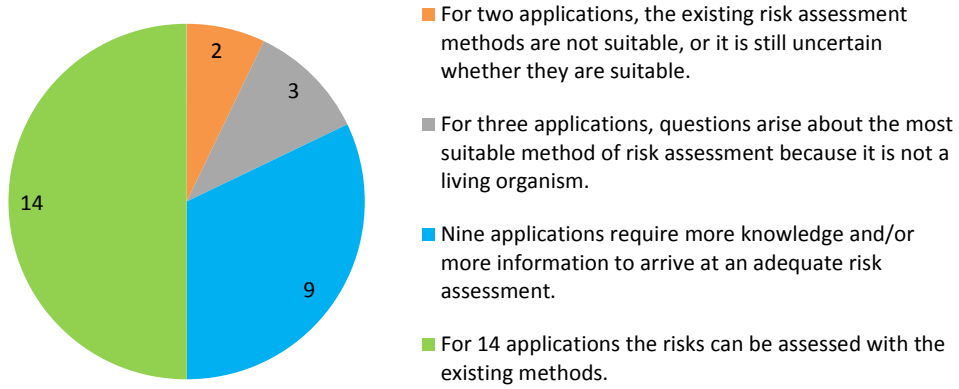

Figure 2: Classification of applications according to assessment category.

Table 1: Overview of the 28 selected applications. The applications are numbered in the left column. This numbering is also used in the remainder of the text. Applications 1 to 13 are used under containment, applications 14 to 28 are used in the environment. One or more examples of each application are given, where possible, and the right-hand column is marked with a colour indicating the outcome of the question structure shown in Figure 1.

#	Description	Examples	Colour (outcome)
Applications under containment			
1	Animal models for studying diseases and developing therapies, genetic modification of animals for other purposes	Animal model in which mutations and deletions are introduced in the genome to study diseases and disease processes, animal model in which multiple genes are inserted to study cancer (multigenetic disease), animal model in which CRISPR/Cas is tested for the treatment of viral infections	
2	Microorganisms with complex new and existing metabolic pathways in closed systems	Yeast with the production route for artimisin, yeast that can break down cell walls of plants for ethanol production	
3	Insects whose genes have been modified	Mosquitoes that can no longer transmit the malaria parasite	
4	Gene drive applications	Synthetic gene drive in an insect, rodent or yeast	
5	Development of therapeutic agents (siRNA, miRNA, antisense oligonucleotides) to treat disorders with aberrant gene expression or viral infections	Preclinical animal models to prevent aberrant gene expression in disorders such as cancer, eye diseases and cardiovascular diseases	
6	EpiEffectors to induce epigenetic changes, fusion	There are many possible clinical applications (cancer treatment, viral and bacterial infections,	

	proteins that influence gene expression through transcription	protein aggregation diseases, metabolic diseases, cellular reprogramming, genetic diseases), but few preclinical models have been developed	
7	Designer chassis, including minimal cells (top-down approach)	Minimal bacteria, minimal yeast chromosomes	
8	Building blocks (the smallest genetic components with a specific function that are used to build genetic circuits)	Kill switch, on and off switch for biosensors	
9	Refactoring (rearrangement of existing, characterised genetic components with the same result)	Glycolysis pathway reorganised and placed at single locus in yeast	
10	Cell-free systems (producing something with cellular machinery, but without using living organisms)	Paper-based diagnostics, in development as a large-scale application	
11	Orthogonal systems (Xenobiology)	Nucleic acids built from new 'letters', alternative protein coding in the DNA, proteins made from new (non-canonical) amino acids	
12	Protocells, non-living	Liposome containing a DNA template and a cell-free extract to produce protein	
13	Protocells, developed into a living cell	*No example is available*	
Applications in the environment			
14	*Ex vivo* therapy (cells, excluding germline cells, are genetically modified outside the body and then reintroduced in the patient)	Deletion of the sequence coding for the HIV receptor in immune cells to make these cells resistant to HIV infection	
15	*In vivo* therapy in somatic cells to treat genetic or infectious diseases in which non-functional or aberrant sequences are repaired or viral sequences are removed	The first applications of gene editing agents in individual patients are now operational, and clinical studies are planned in the USA, for example with ZFN as a weapon against genetic liver diseases	
16	Gene therapy to treat monogenetic diseases in which a non-functional or aberrant sequence is removed or repaired in the germline cells	There are no clinical examples yet, an example of a preclinical application is the correction of mutations in genes that cause hereditary heart disease in pre-implantation human embryos	
17	Algae in semi-closed and open systems	Algae that produce a precursor for plastics, oil or ethanol	

18	Plants modified to influence the microbiome on and around their roots	Plants with altered root exudates	
19	Plants with increased yield due to the association with genetically modified microorganisms	Plants in association with endophytic nitrogen fixing bacteria, or plants treated with disease-suppressing microorganisms	
20	Plants with altered biological characteristics	Plants with efficient nitrogen use, growth rate and/or product yield	
21	Plants with new metabolic pathways	Plants with pathway for nitrogen fixation	
22	Targeted modifications in the genome of livestock or pets	Hornless (polled) cattle or hypoallergenic animals, cattle with inserted genes that can contribute to disease resistance	
23	Modification of the genome of insects	Mosquitoes with progeny that die prematurely or transmit fewer pathogens	
24	Gene drive for population reduction or population modification	Malaria mosquito with offspring that die prematurely, malaria mosquito that cannot transmit the parasite	
25	Clinical application of therapeutic agents (siRNA, miRNA, antisense oligonucleotides) to treat disorders with aberrant gene expression	Treatment of Duchenne (hereditary muscle disease) with oligonucleotide, or other treatment of disturbed expression of genes, for example in cancer, viral infections, eye diseases and cardiovascular diseases	
26	EpiEffectors that can induce epigenetic changes, fusion proteins that influence gene expression through transcription	There are many possible clinical applications, but few preclinical models have been developed. Before clinical application is feasible, many questions regarding patient safety must be answered	
27	RNA construct for gene silencing	Plants with reduced browning, altered flower colour or resistance to diseases or insects	
28	RNA spray	RNA spray to control pest insects or influence plant growth	

3. Analysis and conclusions

For half of the 28 applications analysed in this study, the existing risk assessment method is adequate. For the other half, the existing risk assessment method may not be adequate: for two applications it is still uncertain whether the existing risk assessment method is suitable, for three applications additional questions emerged about the most suitable method of risk assessment (these applications do not involve modification of living organisms), and for about one-third of the applications more knowledge or information is needed to adequately assess the risks.

For all applications, an estimate was made of the period in which these can be expected, based on the available literature and expert judgment. This estimate was then set against the estimated complexity of the modifications to the genetic material or of the application itself. This relationship gives an indication of the urgency of the specific points of

attention for the risk assessment. Results were placed into two groups: applications for use under containment and applications in the environment. The results are shown in Figure 3 and Figure 4.

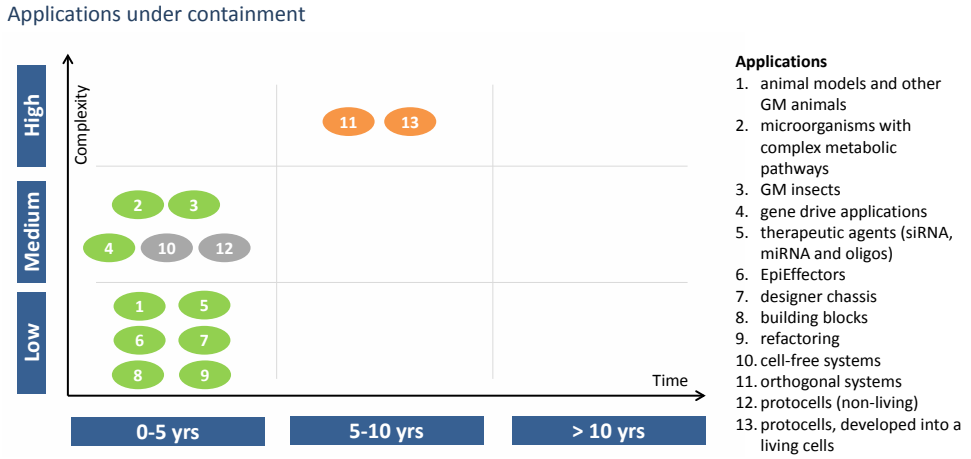

Figure 3: Applications under containment according to the numbering and colour marking of the tables in Section 2. The x-axis shows an estimate of the time period in which the application is expected, the y-axis shows an estimate of the complexity of the modifications to the genetic material or the complexity of the application itself. The estimates of the time period and of the complexity of the applications were divided into three time periods in which the applications are expected: 0-5 years, 5-10 years and 10 years or more: low, medium and high, respectively.

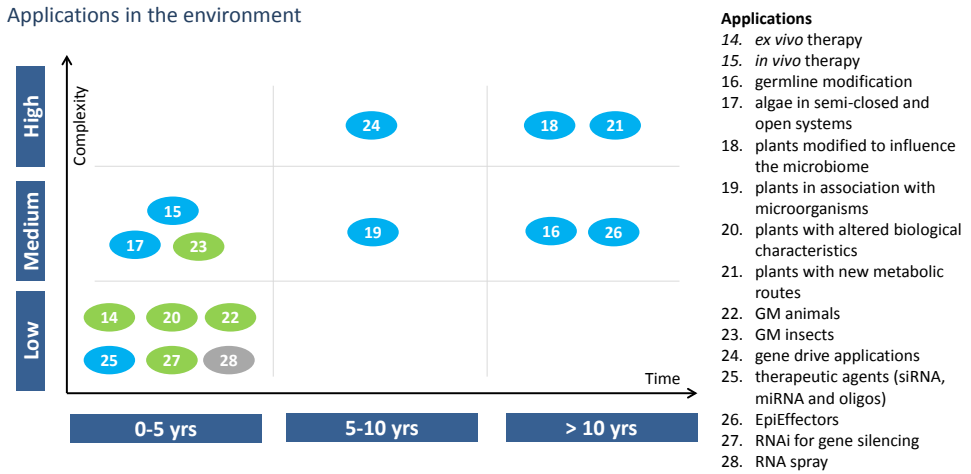

Figure 4: Applications in the environment according to the numbering and colour marking of the tables in Section 2. The x-axis shows an estimate of the time period in which the application is expected, the y-axis shows an estimate of the complexity of the modifications to the genetic material or the complexity of the application itself. The estimates of the time period and of the complexity of the applications were divided into three time periods in which the applications are expected: 0-5 years, 5-10 years and 10 years or more: low, medium and high, respectively.

4. Discussion

The study investigated whether the current assessment method for risks to human health and the environment will continue to be suitable for a wide range of near-future biotechnological applications. The discussion section presents (i) a perspective on actions that can be taken to ensure that adequate risk assessments can be performed for new biotechnological applications, and (ii) several contextual observations.

Action perspective

For the biotechnological applications for which the existing risk assessment method is not adequate – in other words, all applications marked with a colour other than green – the first step is to determine what is necessary to perform an adequate risk assessment, and then to determine what can be done to ensure that risks will also be adequately assessed in the future. This process of translating the conclusions of the research into a concrete action perspective is shown in Table 2.

Table 2: Applications for which the existing risk assessment method might not be sufficient, grouped according to the corresponding requirements and the corresponding action perspective.

Grouped applications	**What is required for an adequate risk assessment?**	**What can be done to fulfil these requirements?**
Applications under containment		
Applications of synthetic biology that do not involve living organisms (10 and 12)	Identify potential adverse effects of cell-free systems and non-living protocells. Determine how these effects can be assessed (with which risk assessment methods or other approaches).	<u>Information:</u> Continue to track developments, both fundamental and application-oriented, in cell-free systems and non-living protocells. <u>Knowledge acquisition:</u> Collect data that provide insight into which potential adverse effects can result from these applications and which questions should be asked in the risk assessment. <u>Knowledge and method development:</u> 1) Survey other risk assessment methods in which adverse effects are identified that are similar to these applications. 2) Build a network of experts who have experience with methods that could be used. 3) If necessary, combine existing risk assessment methods and/or develop a new method.
Applications of synthetic biology for which it is unclear whether the existing risk assessment method is usable (11 and 13)	Knowledge and information is needed to determine 1) the potential adverse effects of orthogonal systems and living protocells on human health and the environment; and 2) whether the GMO risk assessment method is sufficient	<u>Information:</u> Continue to track developments in orthogonal systems and living protocells. <u>Knowledge acquisition:</u> Collect data that provides insight into the various systems and their potential adverse effects. Monitor the extent to which the current GMO risk assessment method remains usable. <u>Knowledge and method development:</u> 1) Build a network of experts. Maintain contact with GMO risk assessment experts to exchange knowledge about the assessment process. 2) If necessary, expand the risk assessment

	or whether other or additional risk assessment questions are required.	method to cover areas for which it currently appears to be unusable.
Applications in the environment		
Applications in red biotechnology for which more knowledge is needed to arrive at an adequate risk assessment (15, 16, 25 and 26)	More knowledge and information is needed about the effects of the agents on humans other than the patient (depending on how the agent is administered). In particular, the first clinical applications will provide information on the safety of relevant agents for the patient, but such data can also be used for assessing possible effects of these agents on humans other than the patient (especially in case of application with viral vectors) and to exclude possible effects on the germline.	Information: - Continue to track developments in clinical applications of these agents, gather information about their *in vivo* effects and monitor developments in the methods of administration and the safety data obtained from studies. - Continue to track developments in the Netherlands, Europe and beyond by maintaining contact with the field of gene therapy research (NVGCT, ESGCT, ASGCT). - Continue to track national and international legislation, regulations and scientific developments with regard to germline modification. Knowledge development: - Intensify contacts with departments within RIVM that deal with epigenetics and environmental assessment of medicines and substances. - Intensify contacts with CCMO, CBG and VWS for sharing knowledge and information about the developments. - Maintain contacts with assessment bodies abroad to exchange experiences in risk assessment.
Applications in green biotechnology that do not concern organisms (28)	Identify risk assessment methods for RNA sprays on plants.	Knowledge and method development: Consult with the Ctgb on the extent to which the risk assessment method (and aspects that are considered in this process) of plant protection products and of GMOs can complement each other when assessing the use of RNA sprays on plants to control insects.
Applications with algae in green biotechnology for which more knowledge is needed to arrive at an adequate risk assessment (17)	More knowledge is needed about the survival and interaction of algae with the environment (water, soil).	Information: Continue to track developments concerning data on GM algae and environmental interactions. Knowledge acquisition: Collect existing reports and risk assessments. Knowledge development: Initiate/maintain contact with authorities who assess applications with GM algae, such as the EPA.
Applications with plants in	More knowledge is needed on the	Information: Continue to track developments regarding effects on the soil ecosystem/soil

green biotechnology for which more knowledge is needed to arrive at an adequate risk assessment (18, 19 and 21)	characterisation of GM plants (in case of introduction of new metabolic pathways), on the determination of potential adverse effects on the soil ecosystem and on methods for determining these effects.	microbiome, with emphasis on functional groups, and targeted methods to measure effects. Knowledge acquisition: Gather existing knowledge (guidelines, reports) on environmental risk assessment of GMOs (plants and microorganisms) and their impact on soil. Knowledge development: Establish/maintain contact with the Ctgb and other authorities in the Netherlands and abroad that have experience with assessing effects of GMOs on soil ecosystems.
Applications with insects for which more knowledge is needed to arrive at an adequate risk assessment (24)	More knowledge is needed to assess possible environmental effects at the population level. The step-by-step principle must be implemented differently, especially for insects with a gene drive.	Information: Continue to track developments in gene drives and their mechanisms and remain linked to the corresponding international network. Knowledge acquisition: Collect data on the environmental introduction of insects with gene drives (naturally occurring or otherwise). Knowledge development: 1) Survey other risk assessment systems for insects such as insects for biological control, insects to control diseases and invasive insect species and how this can contribute to the risk assessment of insects with a gene drive. 2) Establish contact with experts in population dynamics and modelling to explore possibilities for step-by-step introduction into the environment of insects with a gene drive.

The present study focused on 28 applications that together represent the scope and diversity of biotechnology, as expressed in terms of sectors, area of use, or underlying technology. This scope and diversity are good indicators of the innovation potential of biotechnology. The combination of the scope and diversity of biotechnological innovation with the unpredictability of its direction and speed means that innovation must be carefully balanced with safety. The conclusions of this report show that work must continue on developing methodology, gathering and integrating knowledge and acquiring information for the purpose of making adequate risk assessments.

Findings *in context*

The aim of this study was to evaluate the suitability of the existing GMO risk assessment for new biotechnological applications. However, for various applications it was unclear whether they would be covered by current GMO legislation or not.[2] This is in line with the conclusions of COGEM and the Health Council of the Netherlands in the latest Trend Analysis Biotechnology: existing regulations are no longer compatible with the dynamic field of biotechnology, with all the new applications

[2] This situation is illustrated by the interesting case of gene therapy applications that involve deliberate modification of the germline. Such therapy is currently prohibited by the Embryo Act, but that does not address the question of whether risks for offspring of recipients of this germline therapy should be classified and assessed as environmental risks. Algae in a semi-contained facility are another interesting case, as are cell-free systems.

that have recently been developed and are expected in the near future and with convergent technologies.

Biotechnological applications such as modifications to the germline, gene drives or applications of synthetic biology may have a profound impact on our society. For this reason, various scientists have been invited to participate in a societal dialogue on biotechnological innovation.[3] The lower house of parliament in the Netherlands has also called for such a dialogue.[4] In its response[5] to the aforementioned trend analysis, the government has announced that it wants to modernise its policy and regulations on the safety of biotechnology, so that policy and regulation can keep pace with the rapid technological developments. The aim is twofold: to utilise the opportunities offered by biotechnology while ensuring the safety of people and the environment. The societal dialogue is part of this process, and the present study can help to improve that dialogue.

[3] Sheila Jasanoff and Benjamin Hurlbut recently argued that a coordinated international approach is needed to initiate this dialogue. We have noted that work is being done to facilitate such a broad societal dialogue in the Netherlands, at EU level and in a wider international context. See Jasanoff, S. & JB Hurlbut (2018) “A global observatory for gene editing” in *Nature* 555, pp. 435-437.
[4] Parliamentary Paper 27428 No. 340; the Bosma (VVD)/Van der Velde (PvdA) motion in which the coalition government is invited to “initiate a societal debate in which the public would become engaged with current developments in biotechnology”.
[5] Parliamentary Paper 27428, No. 335; Policy response the Trend Analysis in Biotechnology 2016.

Introduction

1.1 Background and aim

Modern biotechnology is a continuation of classical biotechnology (see Section 2.1). Developments in modern biotechnology are succeeding each other at a rapid pace. These developments offer many new possibilities and applications. Some developments, such as genome editing, are a continuation of genetic modification and make it possible to modify the genetic material of organisms more quickly, more efficiently and in a more focused way. Other developments are based on new concepts, such as those in synthetic biology, which centre on the focused design of useful functions in organisms and microorganisms.

Various national and international forums, including COGEM (Netherlands Commission on Genetic Modification) [1], the scientific committees of the European Commission [6] [2], the United Nations Convention on Biological Diversity [3] and the National Academy of Sciences in the USA [4], have signalled that new developments in modern biotechnology raise new issues for risk assessment and have identified knowledge gaps for assessing the risks of these new developments, including those in synthetic biology.

The aim of the present policy report is to determine whether applications of new developments in modern biotechnology can be assessed for risks to human health and the environment using the current risk assessment method for genetically modified organisms (GMOs). This study was commissioned by the Ministry of Infrastructure and Water Management (formerly: the Ministry of Infrastructure and the Environment), which is responsible for the environmental safety of modern biotechnology in the Netherlands.

1.2 Safety of modern biotechnology

When genetic modification became technologically possible, it was still unclear whether GMOs could lead to risks for human health and the environment. The concern was that GMOs could have genetic characteristics, or combinations of characteristics, that had not been seen in an organism before. To protect human health and the environment against potential adverse effects resulting from the use of GMOs, guidelines have been developed to assess these applications and ensure the safe use of GMOs. Initially, these were applications of GMOs under contained conditions, in which the GMOs are kept within a specially equipped facility, for example in laboratories, greenhouses or animal facilities. Subsequently, guidelines and international agreements were developed for GMOs that are released into the environment.

Due to the rapid developments in modern biotechnology and the ever-increasing range of possible applications, it is important to investigate whether the current risk assessment for GMOs can also be used for new applications of biotechnology. If the method developed for GMOs is not

[6] The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Consumer Safety (SCCS).

applicable – or only partly applicable – to these developments, new or additional methods may have to be developed or additional knowledge and information may be needed to adequately perform the risk assessment.

1.3 Approach and scope of this policy report

This policy report provides an overview of new developments in modern biotechnology and examines the consequences of these new developments for assessing their risks for human health and the environment. To gain perspective on this situation, it is necessary to look into the applications of these new developments in more detail. Chapter 2 of this report therefore describes selected examples of new biotechnological applications that can be expected in the next ten years.

In this policy report, the scope of the risk assessment is limited to human health and the environment. In most cases, applications concern organisms or cells. When an organism makes a 'product', for example a chemical, the risk assessment of this product falls outside the scope of this policy report. Risks for human health are defined here as potential pathogenic, toxic or allergenic effects resulting from the biotechnological application. Food safety and patient safety are outside the scope of this report. Given its limited scope, consequences for regulations and other aspects of modern biotechnology, such as biosecurity, are not taken into account in this policy report. For example, security of biological agents and knowledge to prevent misuse is not addressed. The ethical acceptability or socioeconomic aspects of new applications of modern biotechnology also fall outside the scope of this policy report.

Chapter 3 provides a description of the general methodology and the objectives of a risk assessment. After this, the risk assessment method for GMOs is described. The research approach is described in Chapter 4, and Chapter 5 examines whether the risks to human health and the environment of these applications can be adequately assessed using the current risk assessment method, based on 28 selected examples of new biotechnological applications. Chapter 6 ends with the conclusions and provides a short reflection on the research and the action perspective.

2 New developments in modern biotechnology

This chapter provides an overview of the new developments in modern biotechnology. The new biotechnological techniques are categorised and briefly explained. The applications of these techniques in industrial (white), agricultural (green) and medical (red) biotechnology are then briefly described. This overview is based on three exploratory studies on new developments in red, white and green biotechnology [5-7], which RIVM has commissioned, including the reports of COGEM, the scientific committees of the European Commission and NAS. [1, 2, 4, 8, 9].

2.1 What is modern biotechnology?

Biotechnology is literally the technology that is based on biology. It covers very diverse applications, ranging from using bacteria to make cheese to building a synthetic cell. Various phases can be distinguished in the development of biotechnology: classical biotechnology, modern biotechnology and new developments in modern biotechnology. Traditional biotechnology has existed for thousands of years. It includes fermentation processes for the preparation of food and breeding crops and animals (see Figure 5).

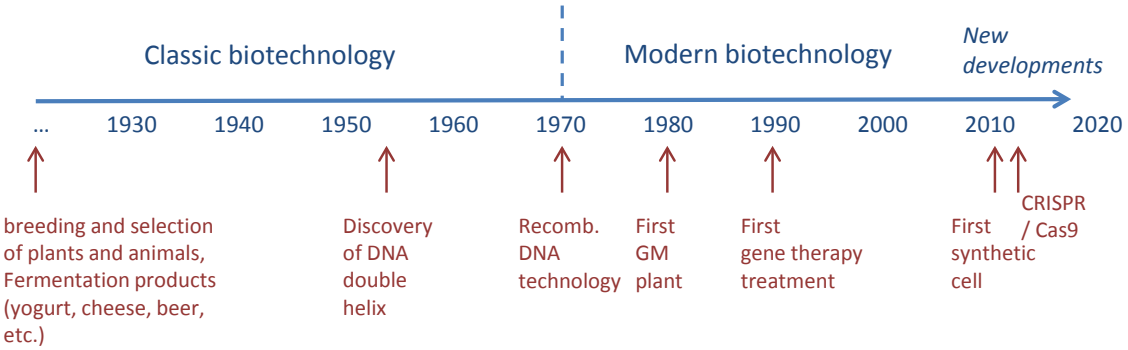

Figure 5: Timeline of biotechnology

Modern biotechnology was introduced around 1970 when it became possible to make changes in DNA, the hereditary material. Genetic modification, which involves combining DNA fragments into new configurations using recombinant DNA techniques, is part of modern biotechnology.

The subsequent phase involves new developments in modern biotechnology. Since the creation of the first organisms with recombinant DNA techniques in the 1970s, modern biotechnology has developed rapidly. For example, the speed of sequencing (reading the sequence of DNA bases) has increased considerably. It has also become possible to make very specific modifications to the genome (for example but using 'genome editing' techniques, such as CRISPR/Cas) or to regulate gene activity without changing the DNA code.
Developments in modern biotechnology are taking place at an increasingly rapid pace. This due not only to the increased knowledge about DNA and biochemical processes, but also to technological

developments such as informatics, bioinformatics and automation. These new developments have led to an increasing range of potential applications in modern biotechnology, including the production of chemicals and medicines, new types of medical diagnostics and therapies, and sensors that can measure environmental pollution.

2.2 Facilitating technologies for modern biotechnology

The available biotechnological toolbox is increasing and becoming more sophisticated. This is due in part to new molecular-genetics techniques (see Section 2.3), but at least as important are technological developments in other areas that are indispensable to advances in biotechnology. This section provides a short description of the most important facilitating technologies that play a decisive role in enabling new developments in modern biotechnology [5-7].

Bioinformatics, software and big data

In bioinformatics, biological knowledge is enriched by analysing biological data. Bioinformatics essentially establishes relationships based on large amounts of data. The developments in this area have a major influence on the possibilities for biotechnology. For example, bioinformatics can be used to identify fragments of comparable DNA, identify proteins with comparable expression patterns or detect genetic abnormalities that occur more frequently in people with a certain hereditary disease. This yields a great deal of information about the function of genes (and proteins) that can be used in biotechnology. This information is becoming increasingly available by storing and providing access to it, for example in databases for protein and DNA sequences, and by developing software to analyse ‘big data’. Software for modelling biological processes is also becoming more advanced and sophisticated. For example, this software uses information from DNA databases to design genes with new functions. Simulation models play an important role in this approach. For instance, models are used to simulate the folding of DNA or RNA (ribonucleic acid) molecules or the metabolic processes in cells.
The developments in information technology are a major driving factor for simulation modelling. The increasing availability of big data on biological processes is facilitated by the growing computing power and storage space to analyse that data.

Robotisation

More labour intensive processes that were previously performed manually in the laboratory, such as cloning and transformation experiments, as well as analysis and selection of mutants and transformants, can now be performed by advanced robotic machinery. Combined with design software, this enables the selection of many modifications in DNA simultaneously, at high speed and at much lower costs.

New sequencing techniques

Sequencing of DNA is the determination of the DNA base sequence (A, T, C and G), and has been used since the 1970s. Sequencing is important for biotechnological applications because it allows for identification of the DNA sequence of genes with specific functions, the

location of genes in the genome, control or modification of the genome, and for design of new metabolic routes. Sequencing techniques develop fast and DNA (and RNA) sequences can be determined more quickly, more accurately, at lower cost and with fewer errors.

Omics
The term 'omics' is used as a collective term for various fields of research in biology. The first field for which the suffix 'omics' was used was genomics. This field aims to sequence the genome of various organisms. Other ‘omics’ fields include transcriptomics (studying messenger RNA – mRNA), proteomics (studying sequences, functions and organisation of proteins), metabolomics (studying the metabolites) and epigenomics (studying epigenetic changes). These fields can provide knowledge about the functioning of cells and organisms that can be used in biotechnology.

DNA and RNA synthesis
DNA can be produced synthetically, just like other chemicals. DNA fragments with a specified base sequence can be ordered commercially. Various fragments of synthetic DNA can then be ‘pasted' together in the right order. This process is called DNA assembly. RNA synthesis is still a lengthy and laborious process during which many errors can occur. But here as well, many technological developments are expected that will greatly simplify and accelerate this process and make it more cost effective.

2.3 New biotechnological techniques and their corresponding applications

This section describes the new biotechnological techniques that play a decisive role in new applications of modern biotechnology. In Section 2.3.1, these techniques are divided into three categories. Based on these categories, Section 2.3.2 provides an overview of the new applications that are expected with the aid of these techniques.

2.3.1 New biotechnological techniques

Based on the mode of action of the technique and its effect on the genome, the new biotechnological techniques are divided into three categories. These are:

- Modification of DNA
- Regulation of gene expression
- Synthetic biology

Below is a brief description of these three categories.

Modification of DNA
Genome editing is the targeted modification of DNA sequences. This can be done by inducing small mutations (small changes of one or more base pairs) or inserting fragments of DNA at specific locations in the genome. Recently, a new method has been developed, known as base editing, which can change a specific nucleotide base (A, T, C or G) into another base without cleaving the DNA [10, 11].

Genome editing uses specific enzymes (site-specific endonucleases) such as CRISPR/Cas (Clustered Regulatory Interspaced Short Palindromic Repeat/CRISPR-associated protein), ZFN (Zinc-Finger Nuclease) and TALEN (Transcription Activator-Like Effector Nuclease). Most applications and developments are currently taking place with CRISPR/Cas whereby increasingly specific Cas proteins are being used [12, 13].

How do genomic editing and base editing work?
Genome editing is performed with 'site-specific nuclease enzymes'. These are composite proteins consisting of one component that binds to a specific DNA region in the genome and a nuclease component (such as the Cas enzyme), which works like molecular scissors and cleaves the DNA (double-strand break) at that specific genomic location. In this way mutations (small changes of one or a few base pairs) can be made at specific locations in the genome. If a DNA sequence is introduced simultaneously with the site-specific nuclease enzyme, this DNA sequence will be integrated into the genome at the cleavage site.
Base editing is based on CRISPR/Cas, but uses 'dead' Cas (dCas) that cannot cleave the double DNA strand, but unfolds it at the right place, thus allowing enzymes to change the nucleotide base [14].

Regulation of gene expression
By regulating gene expression it is possible to influence the expression level of the genes in a cell or organism, without changing the DNA code. In this policy report the term 'regulation of gene expression' is used to indicate that both gene expression and gene regulation can be influenced.

What is gene expression and gene regulation and what is the epigenome?
Gene expression is the process by which the DNA of a gene is 'translated' into the formation of proteins in a cell. This process is mediated by RNA and consists of two steps: converting DNA into mRNA (transcription) and translating mRNA into proteins (translation).
Gene regulation controls gene expression. Specific proteins do not always have to be produced, or are produced in lower quantities. Gene regulation therefore determines the concentration of a protein encoded by a gene in a cell.
The epigenome concerns the entirety of DNA and protein that is folded together as the genetic material in a cell. Epigenetic modifications are changes in gene expression that do not involve changes in the DNA code. Epigenetic changes therefore have no influence on the base sequence of the DNA, but they can be inherited [6, 15].

Gene expression can be influenced by epigenome modification. To this end, genome-editing techniques are used whereby the recognition of a specific DNA sequence in the genome is combined with protein domains that regulate gene expression [15, 16]. Another common technique for influencing gene expression is the use of antisense RNA, also called RNA interference (RNAi) [6, 7].

How does RNAi work?
RNAi is based on double-stranded (ds) RNA (miRNA or siRNA) that is complementary to the mRNA sequence of the gene to be suppressed. The complementary RNA 'sticks' to the relevant mRNA so that the translation cannot take place and the mRNA is degraded. Besides miRNA and siRNA, chemically modified antisense oligonucleotides (ASOs) are also used that can influence gene expression [6].

Synthetic biology
Synthetic biology is 'biological engineering': the synthesis of complex, biology-based (or biology-inspired) systems with functions that do not occur in nature[7] [17]. This category does not consist of specific techniques, but is a conceptual approach that considers biological systems as programmable machines, which can be used to create many new possibilities.

In this approach, knowledge from various fields of research is combined. These fields include molecular biology, cell biology, cell physiology, population genetics, bioinformatics and biochemistry. Synthetic biology is thus a convergent technology, which means that various disciplines and research approaches come together.

Synthetic biology has developed steadily over the past decade. Very diverse applications have become possible, among others due to the engineering-based approach. In this policy report, synthetic biology is divided into four groups of applications (see Section 2.3.5):

- Designer chassis, refactoring and building blocks: constructing, rearranging or building genes using synthesised DNA;
- Xenobiological systems: the use of alternative forms of DNA or amino acids;
- Protocells: the chemical design of components of living cells (to ultimately create life);
- Cell-free systems: in vitro systems with components of cells used to study or mimic cell processes in a simplified environment.

In addition, metabolic engineering – the development and incorporation of metabolic pathways, especially in microorganisms – is often seen as one of the areas of application of synthetic biology. This far-reaching form of DNA modification can be placed under two categories: modification of DNA and synthetic biology. In this policy report, metabolic engineering is placed under the category 'modification of DNA'.

2.3.2 Applications
Sections 2.3.3 through 2.3.5 provide an overview of expected applications in modern biotechnology for each category of techniques, as described in Section 2.3.1(modification of DNA, regulation of gene

[7] Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems, which display functions that do not exist in nature. This engineering perspective may be added at all levels of the hierarchy of biological structures – from individual molecules to whole cells, tissues and organisms. In essence, synthetic biology will enable the design of "biological systems" in a rational and systematic way.

expression and synthetic biology). The time frame in which developments are expected is also indicated. The expected time frame indicates when a risk assessment of these applications will have to be carried out (in the Netherlands).

The overview of applications is not exhaustive and should be seen as a description of several major developments that are possible in the relevant field of application. The overview is based on the three exploratory studies on new developments in red, white and green biotechnology [5-7] and on reports from COGEM, the scientific committees of the European Commission and National Academies of Science [1, 2, 4, 8, 9], among other sources. In the exploratory studies it proved difficult to predict which applications can be expected in the next five to ten years. Therefore, Appendix 1 briefly addresses the most important factors that may determine whether or not certain techniques are expected to be used.

The biotechnological applications are classified per application area: red (medical), white (industrial) and green (agricultural) biotechnology. The boundaries between the various ‘colours’ of biotechnology are vague and sometimes even more colours are distinguished, such as blue (aquatic) biotechnology [18]. However, the scope of this policy report is limited to red, white and green biotechnology. Applications that don’t unequivocally belong to one of these areas are defined as 'other application areas’ in this report. These include certain biotechnological applications in animals or gene drive applications. The applications are described below and are classified on the basis of the three underlying categories of biotechnological techniques described in Section 2.3.1.

2.3.3 Applications involving modification of DNA

Applications in red biotechnology

Gene therapy focuses on the insertion of functional genes to treat a disease or to repair defective genes that cause a disease. Until now, this has usually been done by administering viral vectors or plasmids containing the functional gene, and subsequently integrating them into body cells at random locations in the genome. After this, the functional gene can be expressed. With genome editing, it is possible to integrate or repair/change genes at specific genomic locations. This means that almost every gene can be chosen as a target and that there are many more possibilities for restoring gene function. The success of new therapies depends to a great extent on the efficiency of the delivery systems to bring therapeutic genes into body cells. Most classical gene therapy studies have been performed with viral vectors to deliver the therapeutic genes, but non-viral systems are also being developed and used.

Examples of applications of genome editing in red biotechnology are:

- *ex vivo* therapy, in which somatic body cells (all cells besides germline cells) are removed from the patient and modified outside the body with the aid of genome editing. These cells are then returned to the patient. For example, immune cells can be made resistant to HIV infection [19];
- *in vivo* therapy, in which site-specific nucleases are introduced in the patient's body in order to remove or restore sequences that

cause diseases. Examples are CRISPR/Cas as a weapon against genetic liver diseases [20] and against viral infections such as Hepatitis B virus or Human Papillomavirus [21]; and
- Germline modification, in which non-functional or abnormal sequences are removed or restored in the germ line. For example, a pathogenic mutation in the MYBPC3 gene, involved in a heritable type of heart disease, can be corrected in pre-implantation embryos [22].

The first clinical applications of *ex vivo* therapy using genome editing are expected in the Netherlands within five years [[6]. The development of *in vivo* therapy is also rapidly commencing. The first applications of ZFN have recently taken place in individuals [23, 24] and clinical trials will soon begin in the USA [25]. Consequently, clinical applications are also expected within the Netherlands within five years. Clinical applications of germline modification are not yet foreseen in the Netherlands because this technique is prohibited by law. In addition (apart from ethical discussions) before actual application of human germline modification can take place, the effectiveness and specificity of the modification and safety for the embryo must first be demonstrated[26].

Transgenic animal models are frequently used for clinical research and in development of new gene therapies, including clinical applications of genome editing. Genome editing techniques are also frequently used for the development of new animal models [6].

Applications in white biotechnology
In modern industrial biotechnology, genetically modified microorganisms (bacteria, fungi and yeasts) are used as production organisms. This is done on a large scale in industrial installations, for example for the production raw materials for detergents (enzymes), medicines (e.g. insulin) or food additives (e.g. flavourings).

To optimise production, new metabolic pathways based on synthesised DNA are increasingly introduced into strains of bacteria, fungi or yeast. In this process, several specific modifications in the DNA of the production organism can be made simultaneously. For example, while incorporating new genes into the genome, naturally occurring genes that could disrupt the metabolic process can also be removed. This is called metabolic pathway engineering considered to fall under synthetic biology (see Section 2.3.5). In this policy report, applications of metabolic engineering are categorised under 'modification of DNA'. Metabolic engineering is now being done frequently, and the first applications have already reached the market. One example of such an application is the production of the anti-malaria agent artemisinin by a yeast [27] that has been modified to produce this substance. The applications of metabolic engineering are very versatile, including additives for food products, medicines and products for a biobased economy.

More research is also being done with algae as production organisms. Examples are algae that produce precursors for plastics, biofuel or ethanol [28-30].

Applications in green biotechnology
As in the other application areas, CRISPR/Cas is frequently used in green biotechnology for genome editing of plants [31]. In addition, oligo-directed mutagenesis (ODM) is used, in which targeted mutations in the DNA are realised by using synthetic oligonucleotides [7, 32].

Examples of applications of genome editing in green biotechnology are the following:

- Plants with increased yield due to association with modified microorganisms, such as plants with endophytic nitrogen-fixing bacteria [33], or plants treated with disease-suppressing microorganisms [34-36];
- plants with altered biological characteristics, such as disease-resistant tomatoes [37, 38], herbicide-resistant rapeseed [39], plants with tolerance to abiotic stress such as drought or salt [40]and plants with more efficient nitrogen use or higher growth rate [41, 42];
- plants with new metabolic pathways, such as pathway to fix nitrogen from the atmosphere [43, 44]; and
- plants modified to change their environment, such as plants with altered root exudates to influence the microbiome around the roots [34].

Applications of plants with altered biological characteristics are expected in the next five years. Plants in association with microorganisms will take somewhat longer, probably 6-10 years. Applications such as influencing the microbiome and plants with new metabolic routes will probably take at least ten years. Practical applications of more complex modifications to plant genomes are expected to take even longer.

Other application areas
Animals
Biotechnological applications in animals are very diverse and are mostly grouped under red biotechnology, such as animal models for medical research, but some also fall under other application areas. Examples of applications of genome editing in animals include the following:

- chickens producing hypoallergenic eggs for use in vaccine production or the food industry [45];
- hornless cattle [45];
- genetically modified pigs to grow organs suitable for xenotransplantation [45]; and
- modified insects to prevent the spread of diseases and pests.

Developments in applications involving farm animals mostly take place outside the European Union [45]. The Netherlands has a restrictive policy regarding non-biomedical applications of biotechnology in animals. In the short term (0-5 years), however, it is possible that permits will be requested for certain non-biomedical applications of genome editing in animals.

Gene drives
A special application of genome editing in sexually reproducing organisms is the deliberate incorporation of a ‘gene drive’ system with CRISPR/Cas. Such a synthetic gene drive system is inherited by more

than the usual 50% of the offspring. This enables a trait to spread faster and possibly becomes permanently established in an entire population, even if the trait has no fitness benefit for the organism [46].
Gene drives can be used to change or suppress populations. Quite some applications are possible for the benefit of human or animal health, agriculture and nature protection. Some examples are [47]:

- controlling insect-borne diseases, such as malaria or Lyme disease;
- eliminating invasive exotic species;
- controlling agricultural pests and plant diseases;
- protecting endangered species by making populations resistant to disease or pest organisms.

Research into and development of synthetic gene drive systems is now taking place under contained conditions (in laboratories and insect cages). Applications to introduce gene drive systems into the environment will probably require at least 5-10 years for development and testing.

2.3.4 Applications of regulation of gene expression

Applications in red biotechnology

In medical biotechnology, the possibility of influencing the expression of genes that cause diseases is seen as a clear change as compared to gene repair [6]. These new applications could allow direct and long-term regulation of disease-associated gene expression without modifying the genome.

The most important developments in this area are:

- Application of small therapeutic molecules – such as siRNAs, miRNAs (microRNAs) and antisense oligonucleotides (ASOs) – to influence the regulation of disease-associated genes that are present in body cells. Certain ASOs can also be applied for epigenome modifications. Possible applications, such as the treatment of cancer and infectious diseases, in which aberrant expression of genes plays a role, are now being studied in clinical trials [6];
- Epigenome modification is possible by applying fusion proteins (EpiEffectors) that change the epigenome in a targeted way. This involves, for example, chemical modifications of the DNA or the histones that influence the folding of the DNA and the regulation of gene expression. Although clinical applications are possible, very few preclinical models have been developed for translation to the clinic; many outstanding issues must be addressed to make clinical application possible [15].

Development of the non-viral systems mentioned in Section 2.3.3 also involves these applications. The first clinical applications based on siRNAs and ASOs (mostly applying non-viral delivery systems) are already taking place, but application of EpiEffectors will take longer, perhaps more than ten years [6].

Applications in green biotechnology

In green biotechnology, gene expression in plants or their associated organisms can be influenced by r by RNA produced from an integrated

RNAi construct or with externally applied RNA, for example by using an RNA spray. Some examples are

- RNAi construct: reduced brown colouration in apples, altered flower colour and resistance to diseases or pest insects [48];
- RNA spray: repression of pest insects such as the stalk borer [49] and repression of a virus in bees that could contribute to colony collapse disorder [50].

Application of plants modified with an RNAi construct to make them insect resistant is now taking place in the USA [51] and more applications are expected in the next five years. Important constraints on the further development of RNAi in green biotechnology are the efficiency with which the gene activity can be suppressed, and – for RNAi applications via temporary constructs or RNA sprays – the duration of the effect.

2.3.5 Applications of synthetic biology

The first applications of synthetic biology can be seen in white biotechnology, but more application areas can be expected in the future. Distinguishing between application areas is therefore not useful at present for synthetic biology.

Designer chassis, building blocks and refactoring

A 'chassis' is a basic organism that is often already optimised for the conditions in a bioreactor. With extensive DNA modification, the basic organism can be stripped of undesired or irrelevant genetic information. The resulting basic organism is then called a minimal cell.

'Building blocks' are the smallest genetic components with a specific function that are used to build genetic circuits. Subsequently, these can be introduced into the basic organism, resulting in a designed (or partly designed) organism that has a specific functionality. One example of a building block is a `kill switch' that can induce the death of the microorganism. These kill switches could play an important role in preventing the unintended spread of genetically modified organisms, but more research is needed on their stability and effectiveness [9].
In 'refactoring' an existing genetic code for a complex function of an organism is rewritten and optimised. The genetic code– for example coding for the production of a sugar– can be spread over different parts of the genome. During refactoring, the DNA coding is rearranged, optimised and grouped together with the aim of achieving more modularity [52, 53].

Applications of designer chassis, building blocks and refactoring will be developed over the next five years; the industrial biotechnology sector is currently working with these ideas, concepts and techniques [5].

Xenobiological systems

By using functionally similar– but chemically different – molecular building blocks in the genetic code or during transcription and translation, an alternative, non-naturally occurring biological system (xenobiological or orthogonal system) is created. Examples include the use of other building blocks for the backbone of the DNA, other coding base pairs or an alternative coding for the coded protein. To make this

approach work, the translation machinery of the cell (for example the ribosomes) has to be adapted accordingly. Due to the orthogonality with existing biological systems (the genetic information stored in a xenobiological organism will probably not be interchangeable with that in existing organisms), the corresponding applications are sometimes referred to as having a genetic firewall. Although the developments are still at an early stage, they are seen as important for the development of safe biological systems [54, 55]. The first applications are expected within five to ten years.

Protocells
Protocells are an example of the bottom-up approach in synthetic biology. The aim is to develop simple cell-like systems based on self-developed chemical components. These cell-like systems contain human-designed machinery that can fulfil certain functions [56-58]. One of the long-term goals is to make protocells that are self-replicating. The developments are at a very early stage, so application of living protocells will take at least five to ten years.

Cell-free systems
Cell-free systems use the biological machinery as it occurs in natural cells, but with the cell membrane removed. The system is not self-replicating [59]. Applications include paper-based diagnostics [60]. These applications have already been developed. Industrial applications of cell-free systems as a production platform are in an early stage of development, so these applications will take longer.

2.4 The developments in context

Looking into the future is difficult, but there are strong indications that biotechnology has entered a new phase of development. This is clearly shown by the following indicators:

- Scientific and technological developments are resulting in a rapid pace of discoveries and applications in biotechnology;
- Enabling technologies (such as automation, robotisation, artificial intelligence and omics) shorten the innovation cycle and accelerate product development;
- Abundant investment capital is available, both private and public, for technology development and the development of new applications.
- There is pressure from politicians and policy to use new technology for applications such as the conversion to a low-carbon economy, disease control or health care.

In addition, technologies are converging, which will lead to even more applications. Examples of convergent technologies include bio-nanotechnology and 3D printing with biological materials. Biotechnology is also becoming more accessible for developments outside specialised labs. Both the knowledge and simple tools for conducting biotechnological experiments are now available to everyone.

Based on the above, biotechnology is expected to become an important driving force behind all sorts of technological applications in the foreseeable future. This means that

- more applications will emerge in the near future, and
- these applications will become more complex in nature.

This has potential repercussions for risk assessment and the knowledge required for this assessment.

3 Risk assessment methodology

Certain groups of agents, applications or products are assessed for their potential risks. The aim is to prevent unwanted (adverse) effects on protection goals (see Section 3.1) as a result of using these agents, applications or products. Section 3.2 briefly explains what a risk assessment is and how a risk assessment is performed. Thereafter, the risk assessment method for GMOs is described in more detail (Section 3.3).

3.1 Protection goals

Protection goals are at the basis of risk assessment. They are broadly defined and valued resources, such as biodiversity, ecological functions or human health [61]. They describe the resource that should be protected. Protection goals that focus on biodiversity include preservation of genetic diversity, threatened species (plant or animal), habitats and landscapes. Examples of protection goals focusing on ecological functions are the preservation of soil, water and production systems [61].

Protection goals may differ per country and are often specified in legislation. The goals are usually described in such general terms that they need to be further specified and first translated into specifically formulated values before they can be operationalised in a risk assessment [61].

Examples of protection goals in European legislation on plant protection products, substances and GMOs are, respectively, ‘ensuring a high level of protection of human and animal health and the environment’ (plant protection product legislation) [62], 'a high level of protection of human health and the environment’ ‘(chemicals legislation – REACH) [63] and ‘protecting human health and the environment’ (legislation on genetically modified organisms) [64, 65].

3.2 Risk assessment

A risk assessment is a step-by-step process to assess potential risks of a substance, application or product, such as potential risks to human health or the environment. A risk assessment is based on the basic principle that a risk is determined by the combination of the probability that an adverse effect occurs and the magnitude of the consequences of that effect [66]. In other words, a risk assessment is based on the principle of risk = hazard x likelihood that it may occur. This involves assessing the adverse consequences that an application might have for aspects such as human health or the environment and the likelihood that these consequences will occur. A risk assessment (environmental or otherwise) can be performed both quantitatively and qualitatively. In both cases the potential adverse effects are assessed.

When a risk is identified, measures can be taken to limit the risk; this is known as risk management. Risk assessment and risk management are closely related, but are different processes. During risk assessment

information is collected based on an analysis of the scientific data on the type, size and characteristics of a risk. Risk management involves defining measures to control or limit the risk. These measures are based on the results of the risk assessment. Other factors may also play a role, such as legal, political, social, economic and technical considerations [67].

This methodology is used for the risk assessment of various groups of substances, products, organisms and protection goals. Examples of these groups are chemicals, nuclear radiation, genetically modified organisms, plant protection products, biocides, plant pathogens, animal pathogens, invasive species, protection of workers, patient safety, food safety and consumer safety.

3.3 Risk assessment of genetically modified organisms

Most applications in modern biotechnology concern living organisms whose DNA has been modified. For this group of organisms, the risk assessment method for GMOs is often the most appropriate. This is why the risk assessment of GMOs is described in more detail in this section.

In the risk assessment of GMOs, a distinction is made between applications of GMOs under containment and applications of GMOs that are deliberately released into the environment. The assessment of applications of GMOs under containment focuses on keeping the organism contained and thus preventing contact with the environment. The severity of potential adverse effects as a result of possible contact with the environment determines the level of the containment regime. In contrast, the assessment of deliberate releases of GMOs into the environment focuses on the interaction between the organism and the environment, during which adverse environmental effects must be prevented. Both approaches fall under the umbrella of the risk assessment method for GMOs, which are explained in more detail in Sections 3.3.1 and 3.3.2.

The risk assessment methods for GMOs, the aspects that are taken into account and the basic concepts are comparable worldwide, and are largely based on the work of the OECD [68-70].

The OECD describes two basic concepts for risk assessment of GMOs that are introduced into the environment: the step-by-step principle and the concept of ‘familiarity’ [70]. The step-by-step principle means that in case of great uncertainty about environmental effects, strict risk management measures are taken to limit any risks. As more knowledge is gained about the GMO and its interactions with the environment (familiarity), which indicates that no adverse environmental effects will occur, relatively fewer management measures are required. In this way, GMOs can be introduced into the environment following a step-by-step approach.

The risk assessment method for applications of GMOs under containment, such as in laboratories, greenhouses or animal facilities, is also quite similar worldwide. Several prominent biosafety manuals, such as those published by the National Institute of Health (NIH) from the

USA [71] and the World Health Organization (WHO) [72], describe the basic concepts of biological safety (biosafety) and the classification of microorganisms into four risk groups with associated biosafety levels. GMOs are also included in these manuals.

In Sections 1.3.1 and 1.3.2, the risk assessment methods for GMOs under containment and GMOs released into the environment are described.

3.3.1 Risk assessment for GMOs under containment

The risk assessment for GMOs under containment concerns the risk of potential adverse effects on human health and the environment following unintentional release of the GMO from containment. Containment in this case concerns facilities such as a laboratory, process installation, a specially designed greenhouse or an animal facility. It is examined how activities with a GMO can be carried out safely, under conditions that pose negligible risk to human health and the environment.

The risk assessment begins with the determination of potential adverse effects that may occur as a result of the unintended release of the GMO from containment. Adverse effects include pathogenicity for humans, plants or animals and the exchange of genetic material with other organisms.

The severity of an adverse effect is determined on the basis of the characteristics of the organism and the specific modification. The probability that an adverse effect can occur is determined by the nature of the activities under containment. Both steps (the determination of severity and probability) determine the risk classification of the activity with the specific GMO.

The associated containment level is determined based on the risk classification. After this step the risk assessment is conducted again to determine whether the management measures of the assigned containment level are indeed sufficient to ensure that the risk of the activity with the GMO is negligible. If this is not the case, the management measures will be adjusted. Determination of the correct containment level therefore takes place following an iterative process.

In the case of genetically modified microorganisms used under containment, potential adverse effects are based primarily on the pathogenicity of the GMO for humans, animals and plants. Consequently, the higher the pathogenicity class of the microorganism, the higher the containment level. Furthermore, the potential adverse effects caused by the modification itself are taken into consideration.

For applications other than microorganisms (i.e. animals and plants), the measures are mainly aimed at preventing dispersal of the GMO in the environment. For plants, specific containment measures are used that are related to the reproduction and dissemination method of the plant. For insects, specific measures are prescribed that are aimed at the containment of the insect (for example, the use of mosquito tents and insect traps during activities with insects in an enclosed space).

The general rules and principles for the risk assessment of GMOs under contained use in order to realise the appropriate containment level are described in Figure 6.

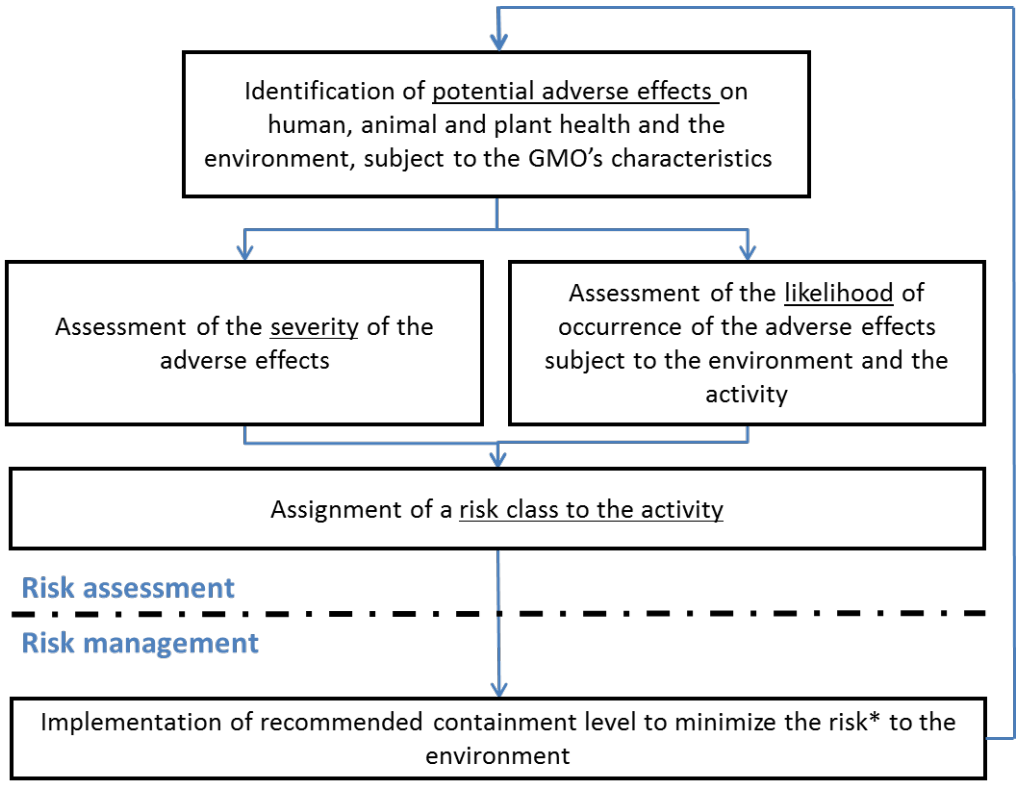

Figure 6: Schematic representation of the risk assessment method for applications involving GMOs under containment.
** Risk = the occurrence of an adverse effect as a result of the dispersal of the GMO from the confined space into the environment*

3.3.2 *Risk assessment for GMOs that are released into the environment*

The risk assessment for GMOs that are deliberately released into the environment is called an environmental risk assessment. In an environmental risk assessment it is determined whether the GMO has potential adverse effects on human health and the environment, both direct or indirect. In this assessment, effects of the GMO are compared with effects of the non-modified parental organism. In this process the same potential adverse effects (areas of concern) are always taken into consideration [73]. Potential risks that are identified can be reduced by risk management measures. These risk management measures are then taken into account in the risk assessment process and the residual risk is thereby determined through an iterative process.

The risk assessment follows the steps described in Figure 7. The assessment starts with a characterisation of the host organism, the genetic modification and the vector used for the genetic modification. The GMO is characterised both on a molecular (genetic aspects) and phenotypic level (traits). Then the interaction of the GMO with the environment into which it is introduced is assessed. This is done by a step-by-step introduction into the environment of the GMOs by means of

field trials, clinical trials or veterinary studies. Due to this stepwise introduction, data can be collected to reduce uncertainties (familiarity), and subsequently risk management measures can be reduced (step-by-step principle). If at any stage potential adverse effects are identified as a result of the environmental introduction of the GMO (compared to an non-modified parental organism), management measures are taken to reduce and control these risks. Finally, the resulting risk is assessed based on the potential adverse effects of the GMO, in combination with the risk management measures. If the risk is considered to be negligible – or acceptable (when other interests are also taken into account) – the application can be authorised.

In gene therapy, a disease can be treated by introducing genetic material into human cells or by modification of existing genetic material. The patient subject to the gene therapy treatment is not part of the environmental risk assessment. In the case of gene therapy, the risk assessment of human health and the environment focuses on the environment of the patient and persons with whom the patient comes into contact.
In somatic gene therapy (including genome modification of somatic cells), the basic principle is that the modification is limited to somatic cells of the patient. In gene therapy involving the germline (germline modification), the therapy can also affect future generations [74].
As part of environmental risk assessments of somatic gene therapies in the Netherlands, besides the risks for human health and the environment (e.g. the possible risks for humans other than the patient in the case of viral vectors), the unintended modification of the germline is also taken into account as potential risk. The outcome of the risk assessment must indicate that this germline modification cannot occur. In the case of gene therapy on the germline, it is unclear whether effects on the offspring of the patient should be taken into account as part of the risk assessment for humans and the environment or as part of the assessment of patient safety.

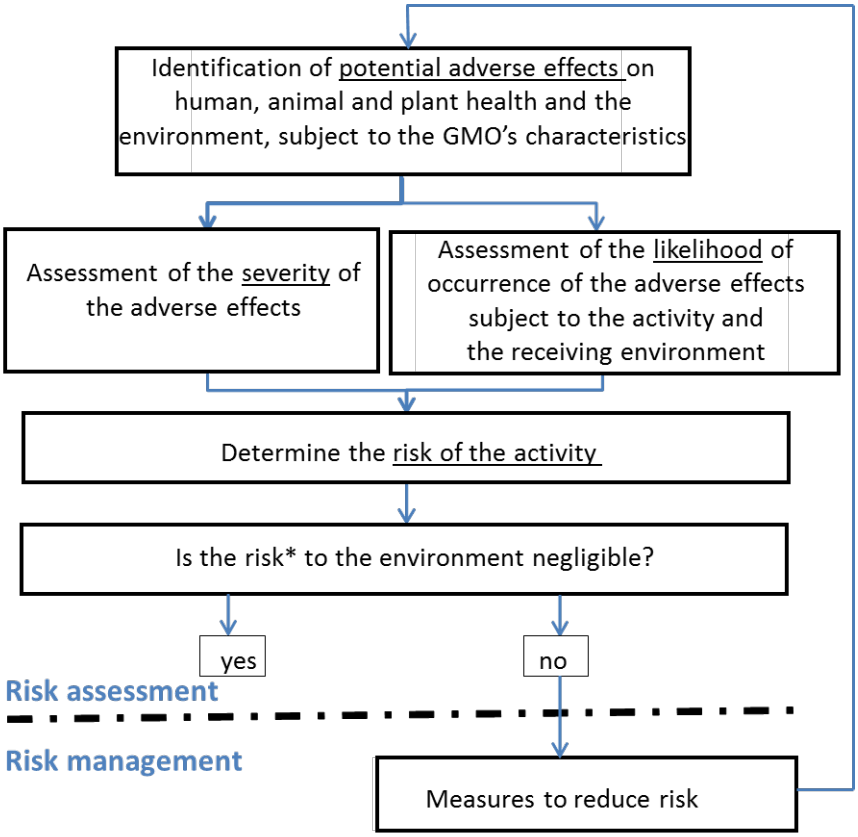

Figure 7: Schematic representation of the risk assessment method for applications of GMOs in the environment.
**Risk = adverse effect on humans, animals, plants and the environment as a result of the deliberate release of the organism into the environment.*
For gene therapy, the risk involves an adverse effect on humans, animals, plants or the environment resulting from the release into the environment of the corresponding organism (e.g. virus, bacteria).

The differences between the two risk assessment methods shown in Figures 6 and 7 are why the new biotechnological applications have been placed into two main groups according to how they will be handled: under containment (4.2) or in the environment (4.3).

4 Research approach

This chapter describes the approach that was used to answer the main research question of this policy report: 'Can applications of new developments in modern biotechnology be assessed for risks to human health and the environment using the current risk assessment method for GMOs?' Section 4.1 discusses the method that was used and Section 4.2 explains the question structure used to answer the research question.

4.1 Method

The research question was answered in five steps. This method is shown schematically in Figure 8.

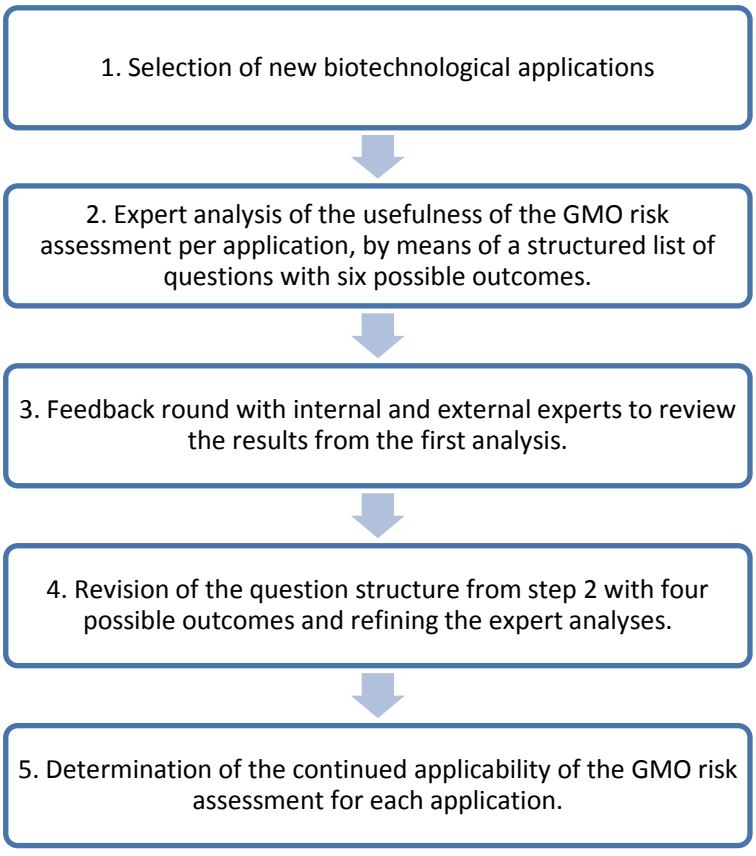

Figure 8: Schematic representation of the steps taken in the research process.

The first step in the process was the selection of new applications in biotechnology. Based on the new biotechnological techniques described in Section 2.3 and the associated applications, the authors compiled a list of 28 applications that are expected to be developed in the next ten years.

In a second step, the possibilities for assessing the risks to human health and the environment were examined for each application. The

risk assessment method for GMOs was used as the starting point because this is considered the most important method for risk assessment of biotechnological applications. Based on expert judgment, the authors estimated the usefulness of the current risk assessment method for GMOs for assessing each of the biotechnological applications. This was done for applications under containment as well as for applications involving release into the environment. In doing so, five questions were systematically asked, with six possible answers (see Appendix 2). Frequent discussions took place during this process to ensure that all experts were answering the questions in a similar way.

The results of the expert assessment were presented to internal and external experts (see Appendix 3 for an overview of the experts involved) to determine whether they came to the similar results (Step 3). Based on the feedback, several aspects of the question structure in step 2 (also shown in Appendix 3) were revised and the number of questions was reduced to four with four possible outcomes (step 4). The adjusted question structure is shown in Figure 9 and is explained in more detail in Section 4.2.

In the last step, the estimates of the continued applicability of the GMO risk assessment for some biotechnological applications were revised (step 5).

The results of this process are presented in Chapter 5.

4.2 Question structure

For each of the 28 new biotechnological applications, the questions in Figure 9 have been addressed. Based on knowledge of and experience with the risk assessment method for GMOs, each application was analysed using expert judgment, which led to one of the four possible outcomes. The questions in Figure 9 are explained below.

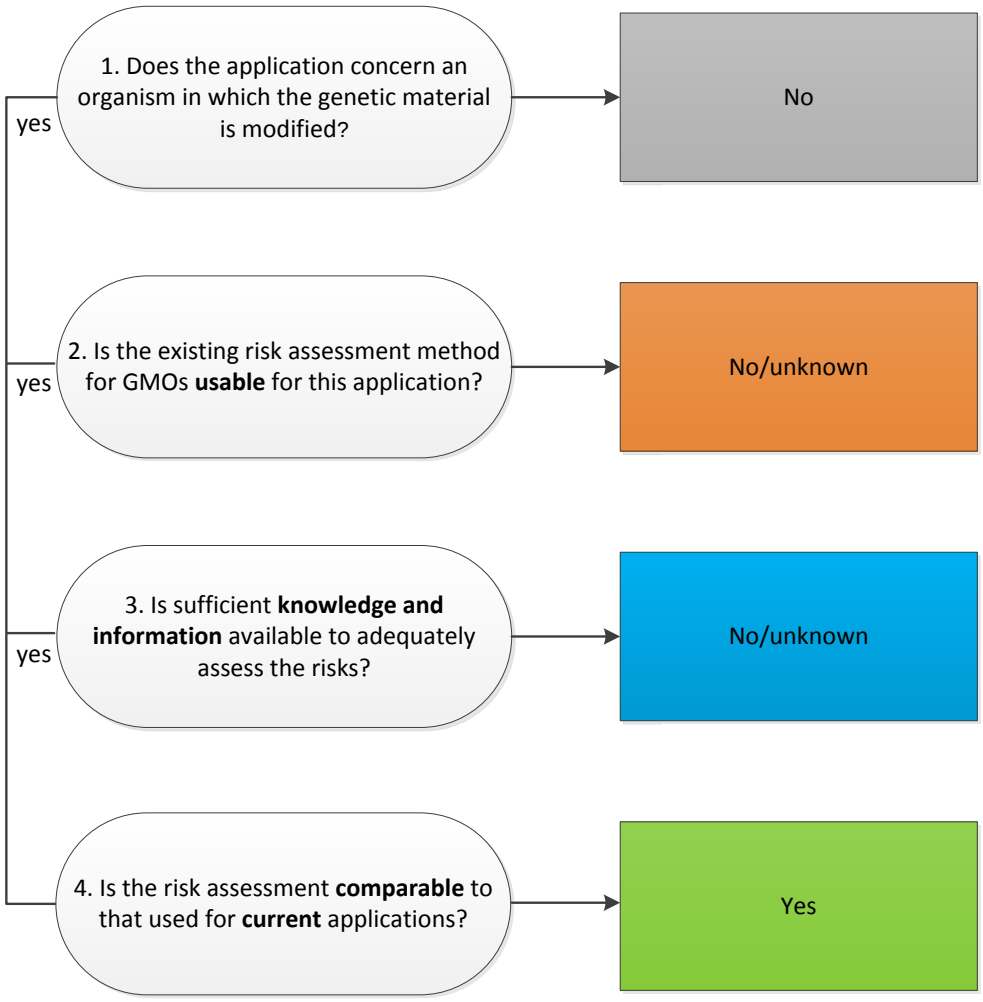

Figure 9: Schematic representation of the question structure that was used when determining whether an assessment can be made of the risks for humans and the environment of new biotechnological applications.

Question 1. Does the application concern an organism in which the genetic material is modified?

The risk assessment method for GMOs was designed for living organisms whose DNA has been modified. In this step, it is checked whether the new application is indeed a living organism in which modifications to the genetic material have been made. In that case, the risk assessment method for GMOs is considered appropriate for assessing the risks to human health and the environment. If it is not a living organism, or if no modification has been made to the hereditary material, the application is marked as grey.

Example
The use of RNA spray to control pest insects or to influence plant growth does not involve the use of an organism (living cells) in which the genetic material is modified. This application is therefore marked as grey.

Question 2. Is the existing risk assessment method usable for this application?
The central question here is whether the risk assessment method as shown in Figures 6 and 7 can be used to assess potential risks of the specific application. This concerns not only the generic way in which the assessment is conducted, but also whether the steps shown in these figures are feasible and sufficient to identify and assess the potential risks.

Example
Protocells that have been developed to become living cells can have components (such as ribosomes, the organelles that translate from RNA to protein) whose functioning differs from that in living cells as we currently understand them. The application of living protocells is still at an early stage of development. Insufficient information is available to assess whether the existing risk assessment method for GMOs can be used for this application. Additional or other adverse effects may be identified, and as a consequence, additional questions could be formulated about the severity of these effects. Therefore, this potential application is marked as orange.

Question 3. Is sufficient knowledge and information available to adequately assess the risks?
An important step in the risk assessment is the characterisation of the new organism. This is a description of the properties of the organism, both molecular and phenotypically. After the organism has been sufficiently characterised, there are two possibilities. In the case of contained use, an assessment is made of the likelihood that the activities with the organism can lead to a adverse effect on the receiving environment as a consequence of an unintentional release. In the case of an environmental application, an assessment is made of the actual interaction of the organism with the corresponding environment and which potential adverse effects may occur. In both assessments, two questions are asked: is there sufficient information to adequately assess the potential adverse effects on the environment and what knowledge is required to actually carry out this assessment?

If not all knowledge and/or information is available, the steps of the risk assessment can still be carried out, but there is a high level of uncertainty. In that case, it is possible to work with assumptions or scenarios and to deploy additional risk management measures to mitigate potential environmental risks to an acceptable level. Additional knowledge and information is then needed to reduce these management measures to a level that is proportional to the actual risk of the application.

Example
An environmental introduction of an organism with a gene drive may potentially result in suppression or modification of an entire population. The consequences of this introduction must be assessed on a population level. Additional knowledge is needed to adequately assess potential environmental risks resulting from modifying or suppressing a population on this (population) level. That is why this application will be marked as blue.

Question 4. Is the risk assessment comparable to that used for current applications?
If questions 1 to 3 can be answered with 'yes', the assessment for this new application is in line with current practice for GMOs. The question can thus be seen as a final check. If this question is also answered with 'yes', this application will be marked as green.

Example
An animal model in which the influence of small molecules (e.g. siRNAs or ASOs) on aberrant gene expression in cancer cells is studied, is a living organism. The method for assessing potential risks for human health and the environment of genetically modified animals under containment has been used for several decades, and is considered to be sufficient to assess this application. Moreover, sufficient knowledge and information is available to determine adequate containment measures to prevent risks for people human health and the environment. This application will therefore be marked as green.

5 Results and analysis

In this chapter the results are described based on the approach taken as described in Chapter 4. In the tables in Sections 5.1 (for applications under containment) and 5.2 (for applications in the environment), the 28 applications are described and the outcome of the question structure is given for each application. These results are analysed in more detail in Section 5.3.

5.1 Applications under containment

5.1.1 Modification of DNA

Table 3: Overview of the applications under containment for the category 'modification of DNA', a possible example of the application and the outcome of the assessment (expert judgment) about whether risks for human health and the environment of the new biotechnological application can be adequately assessed, based on the questions of Figure 9.

No.	Application	Example	Specifics for risk assessment (outcome)
Red biotechnology			
1	Animal models for studying diseases and developing therapies, genetic modification of animals for other purposes	Animal model in which mutations and deletions are introduced in the genome to study diseases and disease processes, animal model in which multiple genes are inserted to investigate cancer (multigenetic disease), animal model in which CRISPR/Cas is tested for the treatment of viral infections	Risk assessment comparable to current GMO applications.
White biotechnology			
2	Microorganisms with complex new and existing metabolic pathways in closed systems	Yeast with the production route for artimisin, yeast that can break down cell walls of plants for ethanol production	Risk assessment comparable to current GMO applications.
Other application areas			
3	Insects whose genes have been modified	Mosquitoes that can no longer transmit the malaria parasite	Risk assessment comparable to current GMO applications.
4	Gene drive applications	Synthetic gene drive in an insect, rodent or yeast	Due to recent research, risk assessment has been sufficiently developed for future applications of organisms with synthetic gene drives.

The current risk assessment method is sufficient for all four applications under containment in which the DNA of an organism has been modified.

For microorganisms that are used as a production organism in white biotechnology (see application 2 in Table 3), a detailed set of criteria is available that is required for risk assessment (see Appendix 6 of the

Netherlands GMO Regulation (Regeling genetisch gemodificeerde organismen milieubeheer 2013)). This approach is also suitable for more complex modifications, such as introducing new metabolic routes, and extensive experience has already been gained in the current assessment practice.

For applications in which the DNA of insects or animals, such as mice, is modified, the existing method of risk assessment is also considered suitable (see applications 1 and 3 in Table 3). The risk assessment focuses on preventing dispersal of the organism. In this area as well, ample experience has been acquired with the risk assessment in the context of GMO risk assessment.

The unintentional release of organisms with a synthetic gene drive can result in the potential change or reduction of an entire population. This has previously been reported on by the RIVM [46, 75] and other institutes. In current practice, the risk assessment for applications under containment is mainly focused on pathogenicity and much less to the potential adverse effect of spreading of the genetic trait, an effect that is actually intended with a gene drive. Due to recent research, the existing assessment method for applications under containment has been sufficiently developed for gene drive applications [76]. Adequate risk management measures can be determined with the available knowledge and information.

5.1.2 Regulation of gene expression

Table 4: Overview of the applications under containment for the category of techniques 'regulation of gene expression', a possible example of the application and the outcome of the assessment (expert judgment) about whether risks for human health and the environment of the new biotechnological application can be adequately assessed, based on the questions of Figure 9.

No.	Application	Example	Specifics for risk assessment (outcome)
Red biotechnology			
5	Development of therapeutic agents (siRNA, miRNA, antisense oligonucleotides) to treat disorders with aberrant gene expression or viral infections	Preclinical animal models to prevent aberrant gene expression in disorders such as cancer, eye diseases and cardiovascular diseases	Risk assessment comparable to current GMO applications.
6	EpiEffectors to induce epigenetic changes, fusion proteins that influence gene expression through transcription	There are many possible clinical applications (cancer treatment, viral and bacterial infections, protein aggregation diseases, metabolic diseases, cellular reprogramming, genetic diseases), but few preclinical models have been developed.	Risk assessment comparable to current GMO applications.

New applications of regulation of gene expression under containment are expected in the medical sector. This concerns the use of antisense RNA (see application 5 in Table 4) or other agents that intervene in the activity of genes in preclinical animal models, usually genetically modified mice (see application 6 in Table 4). The DNA code of the animal is not modified, but some applications may involve epigenetic changes that are inherited. The current risk assessment method for genetically modified animals, which aims to prevent animals from escaping the laboratory, is considered suitable for establishing adequate risk management measures.

5.1.3 Synthetic biology

Table 5: Overview of the applications under containment for the category of techniques 'Synthetic biology', a possible example of the application and the outcome of the assessment (expert judgment) about whether risks for human health and the environment of the new biotechnological applications can be adequately assessed, based on the questions of Figure 9.

No.	Application	Example	Specifics for risk assessment (outcome)
Synthetic biology			
7	Designer chassis, including minimal cells (top-down approach)	Minimal bacteria, minimal yeast chromosomes	Risk assessment comparable to current GMO applications.
8	Building blocks (the smallest genetic components with a specific function that are used to build genetic circuits).	Kill switch, on and off switch for biosensors	Risk assessment comparable to current GMO applications.
9	Refactoring (rearrangement of existing, characterised genetic components with the same result)	Glycolysis pathway reorganised and placed at single locus in yeast	Risk assessment comparable to current GMO applications.
10	Cell-free systems (producing something with cellular machinery, but without using living organisms)	Paper-based diagnostics, in development as a large-scale application	The application does not concern an organism.
11	Orthogonal systems (Xenobiology)	Nucleic acids built from new 'letters', alternative protein coding in the DNA, proteins made from new (non-canonical) amino acids	It is unknown whether the current risk assessment method will be usable for this application.
12	Protocells, not living	Liposome containing a DNA template and a cell-free extract to produce protein	The application does not concern an organism.
13	Protocells, developed into a living cell	*No example is available*	It is unknown whether the current risk assessment method will be usable for this application.

Designer chassis, building blocks and refactoring

For the applications in this subfield of synthetic biology, in which biological systems are engineered (see Section 2.3.5 and applications 7,

8 and 9 in Table 5), the current method of risk assessment is sufficient. In the case of minimal cells, this involves the removal of genetic information from an existing organism; for building blocks, it involves the use of characterised genetic information; for refactoring it involves only a rearrangement of genetic information. Experience has been gained with all these applications in the current risk assessment method of GMOs. This application is therefore marked as green.

Cell-free systems
Cell-free systems (see application 10 in Table 5) consist of genetic components and machinery of living cells, but are themselves not alive. This means that the application does not concern an organism and that the risk assessment for GMOs is not necessarily appropriate for this application. Therefore, this application is marked as grey. Nevertheless, the current risk assessment for GMOs may contain many useful elements because these systems have a similar biological function (i.e. the production of proteins) as a living cell.

Orthogonal systems
Applications such as the use of new building blocks for DNA or proteins, or an alternative coding for translation to protein (see application 11 in Table 5), are still in their infancy. The applications are currently so limited with respect to their intervention in the biology of the organism that the current risk assessment is sufficient. However, in case of further development, it will be necessary to investigate whether the existing method can be used to assess potential adverse effects resulting from organisms that are fully equipped with orthogonal systems. This has also been noted by scientific committees of the European Commission [2]. Additional questions may be required to identify and assess these adverse effects. The application is therefore marked as orange.

Protocells
Non-living protocells (see application 12 in Table 5) can be seen as a collection of chemicals in a closed system. This means that the application does not concern an organism and the risk assessment for GMOs is may therefore not be suitable for this application. However, similar to applications involving cell-free systems, the risk assessment for GMOs may be partly usable because protocells can have a similar biological function (e.g. the production of proteins) as a living cell.

At present there is insufficient insight into how living protocells (see application 13 in Table 5) will be assembled and to what extent these resemble existing organisms. Consequently, it is unclear whether the current method for the risk assessment of GMOs is usable. This application will therefore be marked as orange. The development of living protocells, which is also taking place in the Netherlands, is still in its infancy [77]. Moreover, the scientific committees of the European Commission emphasise that development of knowledge and methods is required to assess potential risks of self-replicating protocells [2].

5.2 Applications in the environment

5.2.1 Modification of DNA

Table 6: Overview of the applications that are released into the environment for the category of techniques 'modification of DNA', a possible example of the application and the outcome of the assessment (expert judgment) about whether risks for human health and the environment of the new biotechnological application can be adequately assessed based on the questions of Figure 9.

No.	Application	Example	Specifics for risk assessment (outcome)
Red biotechnology			
14	*Ex vivo* therapy (cells, excluding germline cells, are genetically modified outside the body and then reintroduced in the patient)	Deletion of the sequence coding for the HIV receptor in immune cells to make these cells resistant to HIV infection	Risk assessment is comparable to current GMO applications.
15	*In vivo* therapy in somatic cells to treat genetic or infectious diseases in which non-functional or aberrant sequences are repaired or viral sequences are removed	The first applications of gene editing agents in individual patients are now operational, and clinical studies are planned in the USA, for example with ZFN as a weapon against genetic liver diseases	More information is needed to characterise the effects in the patient.
16	Gene therapy to treatmonogenetic diseases in which a non-functional or aberrant sequence is removed or repaired in the germline cells	There are no clinical examples yet, an example of a preclinical application is the correction of mutations in genes that cause hereditary heart disease in pre-implantation human embryos	More information is needed to characterise the effects in the patient.
White biotechnology			
17	Algae in semi-closed and open systems	Algae that produce a precursor for plastics, oil or ethanol	More knowledge/information is needed to assess the potential environmental impact.
Green biotechnology			
18	Plants modified to influence the microbiome on and around their roots	Plants with altered root exudates	More knowledge/information is needed to assess the potential environmental impact.
19	Plants with increased yield due to the association with genetically modified microorganisms	Plants in association with endophytic nitrogen-fixing bacteria, or plants treated with disease-suppressing microorganisms	More knowledge/information is needed to assess the potential environmental impact.
20	Plants with altered biological characteristics	Plants with efficient nitrogen use, growth rate and/or product yield	Risk assessment comparable to current GMO applications.

No.	Application	Example	Specifics for risk assessment (outcome)
21	Plants with new metabolic pathways	Plants with route for nitrogen fixation	More knowledge/information is needed to assess the potential environmental impact.
Other application areas			
22	Targeted modifications in the genome of livestock or pets.	Hornless (polled) cattle or hypoallergenic animals, cattle with inserted genes that can contribute to disease resistance.	Risk assessment comparable to current GMO applications.
23	Modification of the genome of insects	Mosquitoes with progeny that die prematurely or transmit fewer pathogens.	Risk assessment comparable to current GMO applications.
24	Gene drive for population reduction or population modification	Malaria mosquito with offspring that die prematurely, malaria mosquito that cannot transmit the parasite	More knowledge/information is needed to adequately assess the potential environmental risks.

Applications in red biotechnology

For gene therapy applications in which human cells (with the exception of germline cells) are modified outside the body (see application 14 in Table 6), the existing risk assessment method is sufficient. In most cases the environmental risks are related to the use of the viral vectors that deliver the therapeutic genes. Much knowledge about and experience with such systems has already been acquired. In addition, there is a tendency to use non-viral vectors that can spread less easily from the patient than viral vectors and for which the environmental risk assessment is relatively less complex. These applications are therefore marked as green.

A major development is the modification of genetic material inside the human body whereby non-functional or aberrant genes are repaired in or removed from somatic cells or cells in the germline (see applications 15 and 16 in Table 6). There are current examples of individual *in vivo* applications of genome editing agents in patients, and the first clinical studies in somatic cells are expected soon. These applications are developing rapidly outside the Netherlands. The first *in vivo* clinical applications in somatic cells are expected in the Netherlands within five years.

For applications in humans, information on patient safety is also important to assess potential environmental risks, particularly when using viral vectors. Genome editing in humans is a relatively new application about and little data is available. Off-target effects of genome editing include unintended modifications of germline cells. Information about potential effects on the germline is difficult to obtain because suitable animal models to test this preclinically are not always available. It is scientifically possible to determine these effects in humans, but there are legal and ethical impediments to carrying out such studies [78]. These applications are therefore marked as blue.

Applications in white biotechnology
There is little experience with genetically modified algae in semi-closed and open systems (see application 17 in Table 6). In these applications, algae are introduced into an aquatic environment, which obviously differs from the environment for terrestrial plants. To make an effective assessment of the consequences for the environment in case these algae are released, it is therefore necessary to develop knowledge about algae as hosts, about the receiving (aquatic) environment, the consequences of possible dispersal and survival, and the effectiveness of containment measures (including biological containment). Algae as a production platform are attracting more and more attention, and knowledge required for risk assessment purposes is growing, but the available knowledge so far is not complete [28, 29, 79, 80]. These applications are therefore marked as blue.

Applications in green biotechnology
For modifications aimed at deliberately influencing the immediate environment of a plant, the risk assessment is more complex. Such an example is a plant genetically modified to influence the microbiome (microbial community) in the soil and on the roots (see application 18 in Table 6). Such microbiomes are very complex. Limited knowledge is available about the complex chemical and biological interactions that take place in the soil microbiome, and little experience has been acquired about actively influencing the soil microbiome [34]. Moreover, little experience has been acquired in assessing the consequences of the new characteristics of the plant on the microbiome. This requires additional information and knowledge development. This application is therefore marked as blue.

For plants with increased yield due to association with modified microorganisms (see application 19 in Table 6), applications are expected at farm level [33]. There is little experience with the introduction of genetically modified microorganisms into the environment and their possible effects on the soil. Although the results of the ERGO research programme [81] have led to more knowledge about the natural variation in microbial soil communities, more knowledge will be needed to identify the potential adverse effects of genetically modified microorganisms on soil functions. Reports on soil interactions in the context of genetically modified plants [82] can be helpful in this regard. This application is therefore marked as blue.

The risk assessment may also be more complex for plants with more extended modifications in the DNA, such as the introduction of a new metabolic pathway or multiple targeted mutations. For example, the introduction of an entirely new metabolic pathway to realise nitrogen fixation (see application 21 in Table 6) requires additional information and knowledge to arrive at a well-founded risk assessment. The current method therefore does apply, but an adequate risk assessment will require additional information and knowledge. There are two underlying reasons for this: 1) the introduction of a new metabolic pathway can interfere with other metabolic pathways in the plant, and 2) the changes induced in the plant may have consequences for the interaction with the specific environment to which the plant is introduced, such as possible adverse effects on the soil ecosystem in which the plant is grown.

For plants modified in a way that is comparable with classical genetic modification, the risk assessment process is also similar to the current approach for GMOs (see application 20 in Table 6). Because the modification is relatively simple, the characterisation of the organism in general is also simple; therefore the current risk assessment is sufficient. For the assessment of the plant's interaction with the specific receiving environment, the current approach and implementation for risk assessment is also sufficient. Much experience has already been gained with genetically modified plants [83, 84]. This application is therefore marked as green.

Other application areas

For targeted modifications of the genome in large farm animals (see application 22 in Table 6), the characterisation of the organism is relatively simple. The step-by-step principle is sufficient to identify possible consequences for the environment. Management and containment measures for large farm animals are relatively simple to implement; as a result the animals can be introduced into the environment in a stepwise approach. Off-target effects can be identified, for example, by sequencing the entire genome. This application is therefore marked as green.

In the risk assessment method for applications in the environment, the 'step-by-step' principle (phased introduction) is an important concept that has been developed for plants in particular [68]. For insects such as mosquitoes, the phased introduction into the environment is more difficult. This is because insects, such as mosquitoes, can disperse freely (see application 23 in Table 6). For this type of organism, the step-by-step principle must therefore be applied differently than for plants. For applications such as mosquitoes, the existing risk assessment method is still adequate. This application is therefore marked as green.

For assessing the consequences for the environment of, for example, mosquitoes with synthetic gene drive systems (see application 24 in Table 6), more knowledge must be acquired to adequately assess risks. Because the genetic intervention is aimed at modifying populations or subpopulations, the effects can theoretically affect large geographical areas, and can occur more quickly and on a larger scale (at the population level). There are many questions about the risk assessment and control strategies, in particular about how the consequences of a modified population can be assessed at the ecosystem level [46, 47, 75]. Other assessment methods, such as those for invasive species that have established themselves outside their original range and could therefore pose a threat to biodiversity, could provide new insights for the risk assessment of gene drive applications. The step-by-step principle also requires an alternative approach for organisms with a synthetic gene drive system. This topic is receiving much international attention, e.g. in the Ad Hoc Technical Expert Group (AHTEG) on synthetic biology [85].

5.2.2 *Regulation of gene expression*

Table 7: Overview of the applications that are released into the environment for the category of techniques 'regulation of gene expression', a possible example of the application and the outcome of the assessment (expert judgment) about whether risks for human health and the environment of the new biotechnological application can be adequately assessed based on the questions of Figure 9.

No.	Application	Example	Specifics for risk assessment (outcome)
Red biotechnology			
25	Clinical application of therapeutic agents (siRNA, miRNA, antisense oligonucleotides) to treat disorders with aberrant gene expression	Treatment of Duchenne (hereditary muscle disease) with oligonucleotide, or other treatment of aberrant expression of genes, for example in cancer, viral infections, eye diseases and cardiovascular diseases	For certain applications that could lead to epigenetic changes, more knowledge/information is needed.
26	EpiEffectors that can induce epigenetic changes, fusion proteins that influence gene expression through transcription	There are many possible clinical applications, but few preclinical models have been developed. Before clinical application is feasible, many questions regarding patient safety must be answered	For certain applications, more knowledge/information is needed.
Green biotechnology			
27	RNA construct for gene silencing	Plants with reduced browning, different flower colour or resistance to diseases or insects	Risk assessment comparable to current GMO applications.
28	RNA spray	RNA spray to control pest insects or influence plant growth	This does not involve a living organism whose DNA is modified.

Applications in red biotechnology

Risks of medical applications of agents that influence gene expression without changing the DNA code (applications 25 and 26 in Table 7) are often exclusively patient-related and not environment-related. For certain applications, an environmental risk assessment could also be relevant if viral vectors are used to administer the agents that influence gene expression. Relevant information obtained in the context of patient safety is often used in the environmental risk assessment to estimate the possible effects in case of exposure of humans other than the

patient. Besides effects on the patient, there are also potential effects of agents that influence gene expression in the germline and in offspring [6]. Effects of substances on the germline and on offspring are usually investigated by means of multigenerational research with laboratory animals. However, the extrapolation of animal data to humans is sometimes difficult and data on for instance possible effects later in life due to prenatal and early postnatal exposure are difficult to obtain [86]. Such information is important to patient safety, but possibly also for the assessment of the risks to human health and the environment. These applications are therefore marked as blue.

Applications in green biotechnology

For applications in which gene expression is deliberately influenced with a construct that ensures the production of antisense RNA (see application 27 in Table 7), the risk assessment is comparable to that for current GMO applications. The RNA that affects gene expression is produced by the plant and ensures the targeted suppression of its own genes or genes of herbivorous insects. This application is therefore marked as green.

Although the risk assessment of such plants is similar to that for current applications, some tests may require a different approach, such as toxicity testing for non-target insects.

RNA can also be used as a spray to suppress the expression of genes in the plant or in insects eating the plant (see application 28 in Table 7). This application consequently contains no living organism, but only RNA. This application is therefore marked as grey. The risk assessment for plant protection products may be to be more appropriate for this application. The risk assessment for GMOs is also usable in part for this application.

5.3 Analysis of the results

The results as described in Sections 5.1 and 5.2 provide an overview of the individual applications for which challenges may be expected in the risk assessment. In this section further analysis is performed to provide a more general picture of the adequacy of the risk assessment of GMOs for new biotechnological applications. To this end, the applications are classified according to the period when a risk assessment is expected to be required and according to the complexity of the intervention and/or the application. These two aspects are explained below. The classification is only an indication and is based on an estimation of the authors.

Time

Section 2.3 provides an indication of when the new biotechnological applications are expected. The expected time frame indicates when a risk assessment for these applications will have to be carried out (in the Netherlands). This provides an idea of the urgency of the actions to be undertaken to acquire knowledge for/experience with the risk assessment of specific applications.

In this policy report, a qualitative estimate has been made of the most important developments in biotechnology that can be expected in the next ten years. For a few applications, it is expected that it will take

more than ten years before a risk assessment will have to be carried out (in the Netherlands).

Predicting the term during which applications are expected is very difficult because this depends on many factors (see also the drivers and barriers for developments described in Appendix 1). This is discussed in more detail in Section 6.2.

Complexity
A general trend observed in the biotechnological applications addressed in this policy report is the increasing complexity of the interventions in the genome or epigenome. The applications themselves are also becoming more complex and more diverse. Both of these developments have consequences for the risk assessment. In many cases, more complex interventions in organisms and more complex applications require a more extensive and complex risk assessment, which often requires more knowledge and information.

An additional problem for performing risk assessments on radically modified organisms is that it is more difficult to compare the modified organism with the parental organism (comparator). To solve this problem, multiple comparators can be used, which do not necessarily have to be the wild-type parental organism, but this could require re-evaluation or adaptation of the assessment. Assessment without the use of a comparator is theoretically possible, but may require a large amount of information, which is complex and time-consuming.

For applications that are introduced into the environment, the increasing complexity of the applications makes it more difficult to assess the resulting interactions with the environment:

- the interactions with the environment are more difficult to predict;
- new applications may relate to other receiving environments that have not previously been considered; and
- applications can lead to other adverse effects, which until now were evaluated at a lesser extent (e.g. effects of gene drives at the population level).

5.3.1 Applications under containment

Figure 10 shows an estimate of the period in which the new biotechnological applications under containment can be expected, compared with the complexity of these applications. This gives an indication of the urgency of specific points of attention in the risk assessment.

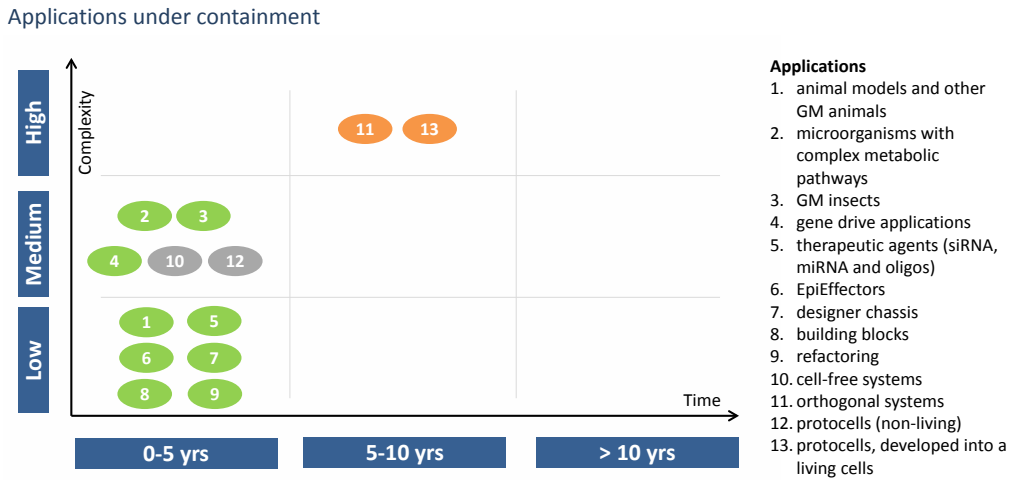

Figure 10*: Applications under containment according to the numbering and colour marking of the tables in Chapter 4. The x-axis gives an estimate of the time period in which the application is expected, the y-axis gives an estimate of the complexity of the modifications to the genetic material or of the application itself. The figure is divided into three time periods: short term (0-5 yrs.), medium term (5-10 yrs.) and long term (> 10 yrs.) and three levels of complexity (low, medium, high). The classification of the applications involves expert judgment based on current developments. The position of an application within each box has no significance.*

For almost all applications that are expected in the short term (the coming 0-5 years), it is estimated that the risk assessment is comparable to that which is used currently for GMOs. This means that the risk assessment for these applications is in order.

Cell-free systems and protocells (non-living) are an exception. These applications do not involve living organisms. For these applications, the risk assessment for GMOs is therefore not the most appropriate method. For both types of applications, the questions posed in the risk assessment for GMOs might be partly appropriate: the applications do have similarities with organisms. In this study, we did not investigate whether other risk assessment methods would be better suited or more complete for these applications. Different methods could potentially be combined to ask the right questions. This requires integration of knowledge from various risk assessment methods. Given the short term in which these applications are expected, further research into this topic should have a high priority. The scientific committees of the European Commission have also identified the use of non-living protocells as one of the knowledge gaps for the risk assessment of applications in synthetic biology. Among other things, they have called for more attention for the consequences of cell-cell interactions between non-living protocells and living cells [2].
In the medium term (5-10 years), applications are expected (such as orthogonal systems and protocells developed into living cells) for which it is still unclear whether the existing risk assessment method is usable. This not only has to do with uncertainty about how the biological machinery of these applications will take shape, but also with the fact that these applications can be so different from the living cells that it is

unclear whether the appropriate questions are asked in the risk assessment.

5.3.2 *Applications in the environment*

Figure 11 shows an estimate of the period in which the new biotechnological applications that are introduced into the environment can be expected, compared with the complexity of the risk assessment for these applications. This gives an indication of the urgency of specific challenges in risk assessment.

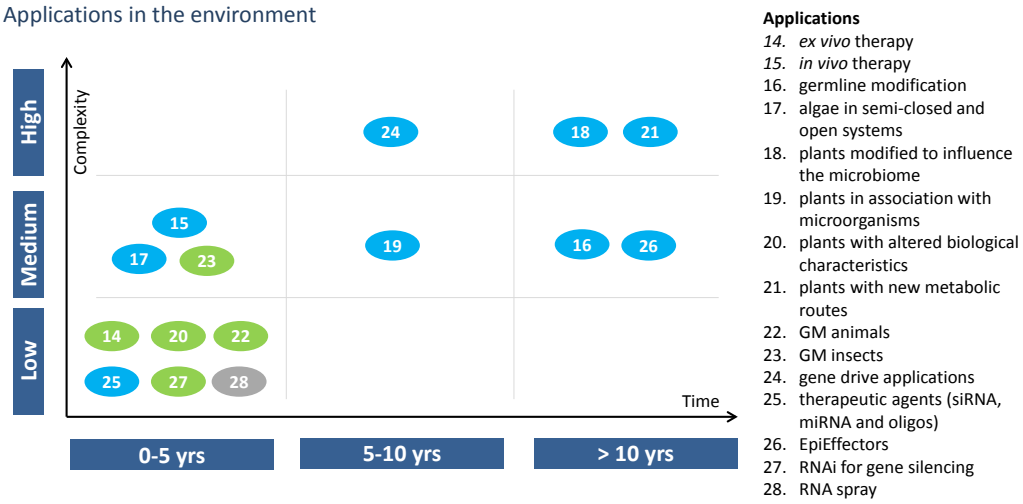

Figure 11: Applications in the environment according to the numbering and colour marking of the tables in Chapter 4. The x-axis gives an estimate of the time period in which the application is expected, the y-axis gives an estimate of the complexity of the modifications to the genetic material or of the application itself. The figure is divided into three time periods: short term (0-5 yrs.), medium term (5-10 yrs.) and long term (> 10 yrs.) and three levels of complexity (low, medium, high). The classification of the applications involves expert judgment based on current developments. The position of an application within each box has no significance.

The figure for applications that are introduced into the environment (Figure 11) shows similarities with the figure for applications under containment (Figure 10), but there are also some differences.

One similarity is that a number of applications are expected in the near future for which the risk assessment is comparable to that for current applications. For these expected applications, the current risk assessment is in order. However, we also see an application (RNA spray) that does not involve a living organism. For this application, the risk assessment for GMOs may therefore not the most appropriate method, although it can be useable in part. As this application concerns a plant protection product, the risk assessment for this group of substances may probably be the most appropriate method. Combining the different methods may give an added value.

The graph for new applications that are introduced into the environment clearly shows a different picture than that for applications under containment with regard to the knowledge and/or information required

to adequately assess potential risks. In both the short and medium term, applications are expected for which insufficient knowledge and/or information is available to arrive at an adequate risk assessment. To perform an adequate risk assessment, more information must be collected and more knowledge must be developed. Some of the required knowledge is already available elsewhere and will have to be compiled (knowledge integration). However, some of the knowledge will still have to be acquired through additional research.

6 Conclusions and discussion

In this chapter, conclusions are drawn from the results and analysis obtained so far. Regarding new applications for which the risk assessment is not comparable to that for current applications, the necessary steps to be able to adequately risk assess potential risks are specified. To conclude the chapter, the conclusions are placed in a broader context.

6.1 Conclusions of the study

In this study the consequences of new applications of developments in modern biotechnology for assessing the risks to human health and the environment were investigated. These are the conclusions (see also Figure 12 for how the conclusions relate to the various applications):

The risk assessment is adequate for half of the new biotechnological applications selected for this study.
Of the 28 new biotechnological applications that were selected, 14 are expected in the next five years. For this group the risk assessment was found to be comparable to that for current applications of GMOs. The risk assessment method for GMOs is the most appropriate for this group because these new applications all concern living organisms whose genetic material has been modified. Moreover, the current assessment method is suitable to use for assessing these applications, and sufficient knowledge and information is available to enable an adequate risk assessment.

In the short term, biotechnological applications are expected for which the risk assessment for GMOs may not be the most appropriate method.
Three of the new biotechnological applications do not concern living organisms. These applications are all expected in the next five years. For these applications, the risk assessment method for GMOs may not be entirely suitable. Therefore it should be investigated whether other risk assessment methods may be more suitable for these applications, or whether it is necessary to combine aspects from different risk assessment methods to come to a more adequate assessment. Given the short term in which these applications are expected, further research into this topic should have a high priority.

In the medium term (5-10 years), applications are expected for which it is still unclear whether the existing risk assessment method is usable.
Two applications in synthetic biology are at such an early stage of development that it is currently unclear how these will take shape and what their potential adverse effects could be for human health or the environment. When more is known about the nature of the applications, it can be established whether the risk assessment method for GMOs is adequate or whether it should be adapted for these applications.

Is additional knowledge and information required to arrive at an adequate risk assessment?
For nine applications that will be introduced into the environment, additional knowledge and information is needed. In both the short and longer term, this is the biggest constraint for an adequate assessment of the risks for human health and the environment of these new biotechnological applications. Due to the broader range of applications and the increased complexity of the corresponding interventions, the implementation of the risk assessment requires information acquisition, knowledge development and knowledge integration.

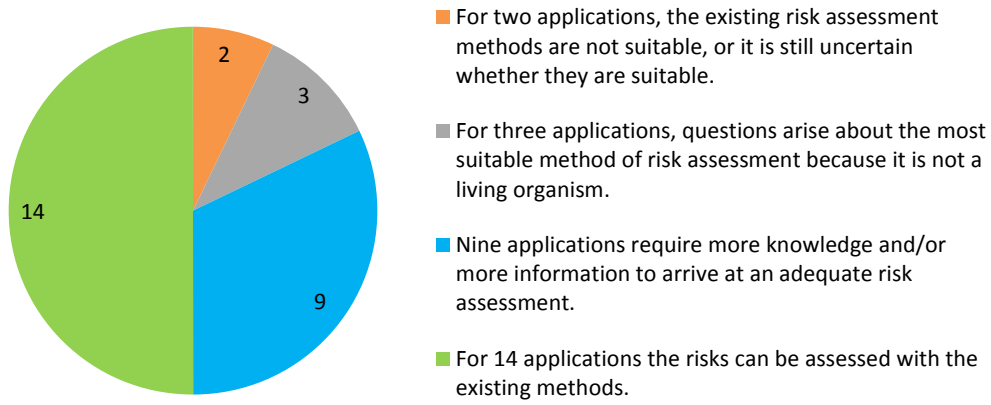

Figure 12: Conclusions on the suitability of the existing risk assessment method for the 28 new biotechnological applications.

6.2 Discussion

Considerations

The research described in this policy report was carried out in response to a question from the Ministry of Infrastructure and Water Management about applicability of the existing risk assessment for future biotechnological applications. This question resulted from the rapid pace of developments in biotechnology. Prompt anticipation of these developments is important because it takes time to identify gaps in the existing knowledge and risk assessment methods and to develop new knowledge and methods to fill these gaps.

The 28 new biotechnological applications that were selected for this study cover a non-exhaustive but broad range of applications. Due to factors that cannot be anticipated, the speed and direction of the actual developments may differ from those assessed in this report. For example, when influential actors perceive that they have an important interest in a certain development (such as a drug to treat a serious, common medical condition), the development can accelerate. This is impossible to predict. The expected time period for the applications that we present in this policy report must therefore be seen as a rough indication.

The research described in this policy report is based largely on expert judgment. The authors' appraisals largely determined the outcomes of this policy report, but a review by internal and external reviewers (see

Appendix 2 for an overview of their expertise) supported these outcomes. The feedback from the internal and external reviewers also helped to simplify the question structure that was used for the study, which was reduced from five questions with six possible outcomes to four questions with four possible outcomes. According to the reviewers, the additional specification of questions and outcomes in the initial question structure led to ambiguity, and their feedback showed that this specification was not required for the study. This feedback therefore helped to structure the questions in a more efficient way.

This policy report can be seen as first inventory of the relevance of the current risk assessment for new biotechnological applications. One of the next steps may be to investigate whether and how other assessment methods could be used or combined. Gaining insight into this step requires the acquisition of broader expertise.

Action perspective

Section 6.1 showed that 14 of the 28 new biotechnological applications raise points of attention with respect to the ability to adequately assess the risks for human health and the environment. To be able to perform an adequate risk assessment for these applications, additional research is needed into the risk assessment method or more knowledge and information must be acquired. Here we discuss what could be done in the Dutch context to contribute to this process.

The points of attention identified in this policy report are not inherent to the Dutch situation; they are also important in an international context. The risk assessment method for GMOs has a comparable basis internationally (see Chapter 3). This means that the research questions and knowledge gaps do not always have to be answered/filled in at the national level. Indeed, operating in an international context provides added value.

Table 8 below provides an overview of which elements are necessary to arrive at an adequate risk assessment and which steps can be taken to achieve this.

Table 8: Overview of possible actions to address the points of attention identified in the risk assessment for grouped applications. The numbering of the applications used in this policy report is shown in brackets.

Grouped applications	**What is required for an adequate risk assessment?**	**What can be done to fulfil these requirements?**
Applications under containment		
Applications of synthetic biology that do not involve living organisms (10 and 12)	Identify potential adverse effects of cell-free systems and non-living protocells. Determine how these effects can be assessed (with which risk assessment methods or other approaches).	Information: Continue to track the developments, both fundamental and application-oriented, in cell-free systems and non-living protocells. Knowledge acquisition: Collect data that provide insight into which potential adverse effects can result from these applications and which questions should be asked in the risk assessment. Knowledge and method development: 1) Survey other risk assessment methods in which adverse effects are identified that are similar to these applications. 2) Build a network of experts who have experience with methods that could be used. 3) If necessary, combine existing risk assessment methods and/or develop a new method.
Applications of synthetic biology for which it is unclear whether the existing risk assessment method is usable (11 and 13)	Knowledge and information is needed to determine 1) the potential adverse effects of orthogonal systems and living protocells on human health and the environment; and 2) whether the GMO risk assessment method is or whether other or additional risk assessment questions are required.	Information: Continue to track developments in orthogonal systems and living protocells. Knowledge acquisition: Collect data that provides insight into the various systems and their potential adverse effects. Monitor the extent to which the current GMO risk assessment method can beused. Knowledge and method development: 1) Build a network of experts. Maintain contact with GMO risk assessment experts to exchange knowledge about the assessment process. 2) If necessary, expand the risk assessment method to cover areas for which it currently appears to be unusable.
Applications in the environment		
Applications in red biotechnology for which more knowledge is needed to arrive at an adequate risk assessment	More knowledge and information is needed about the effects of the agents on humans. In particular, the first clinical applications will provide information on the safety of relevant	Information: - Continue to track developments in the clinical applications of these agents, gather information about their *in vivo* effects and monitor developments in the methods of administration and the safety data obtained from studies. - Continue to track developments in the Netherlands, Europe and beyond by maintaining contact with the field of gene therapy research (NVGCT, ESGCT, ASGCT).

Grouped applications	What is required for an adequate risk assessment?	What can be done to fulfil these requirements?
(15, 16, 25 and 26)	agents for the patient, but such data can also be used for assessing possible effects of these agents on humans other than the patient (especially in case of application with viral vectors) and to exclude possible unintended effects on the germline.	- Continue to track national and international legislation, regulations and scientific developments with regard to germline modification. Knowledge development: - Intensify contacts with departments within RIVM that deal with epigenetics and environmental assessment of medicines and substances. - Intensify contacts with CCMO, CBG and VWS for sharing knowledge and information about the developments. - Maintain contacts with assessment bodies abroad to exchange experiences in risk assessment.
Applications in green biotechnology that do not concern organisms (28)	Identify risk assessment methods for RNA sprays on plants	Knowledge and method development: Consult with the Ctgb on the extent to which the risk assessment method (and aspects that are considered in this process) of plant protection products and of GMOs can complement each other when assessing the use of RNA sprays on plants to control insects.
Applications with algae in green biotechnology for which more knowledge is needed to arrive at an adequate risk assessment (17)	More knowledge is needed about the survival and interaction of algae with the environment (water, soil)	Information: Continue to track developments concerning data on GM algae and environmental interactions. Knowledge acquisition: Collect existing reports and risk assessments. Knowledge development: Initiate/maintain contact with authorities who assess applications with GM algae, such as the EPA.
Applications with plants in green biotechnology for which more knowledge is needed to arrive at an adequate risk assessment (18, 19 and 21)	More knowledge is needed on the characterisation of GM plants (in case of introduction of new metabolic pathways), on the determination of potential adverse effects on the soil ecosystem and on methods for determining these effects.	Information: Continue to track developments regarding effects on the soil ecosystem/soil microbiome, with emphasis on functional groups, and targeted methods to measure effects. Knowledge acquisition: Gather existing knowledge (guidelines, reports) on environmental risk assessment of GMOs (plants and microorganisms) and their impact on soil. Knowledge development: Establish/maintain contact with the Ctgb and other authorities in the Netherlands and abroad that have experience with assessing effects of GMOs on soil ecosystems.
Applications with insects for which more	More knowledge is needed to assess possible environmental	Information: Continue to track developments in gene drives and their mechanisms and remain linked to the corresponding international network. Knowledge acquisition: Collect data on the

Grouped applications	What is required for an adequate risk assessment?	What can be done to fulfil these requirements?
knowledge is needed to arrive at an adequate risk assessment (24)	effects at the population level. The step-by-step principle must be implemented differently, especially for insects with a gene drive.	environmental introduction of insects with gene drives (naturally occurring or otherwise). Knowledge development: 1) Survey other risk assessment systems for insects such as insects for biological control, insects to control diseases and invasive insect species and how this can contribute to the risk assessment of insects with a gene drive. 2) Establish contact with experts in population dynamics and modelling to explore possibilities for step-by-step introduction into the environment of insects with a gene drive.

Looking beyond the existing framework

The technical possibilities and applications of biotechnology are becoming increasingly complex and broader. The requirements listed in Table 8 for continuing to assess risks in an adequate way, indicate that risk assessment research should also be broadened. It is important to look beyond the existing framework of the risk assessment for GMOs.

The first aspect concerns broadening the risk assessment method as used for GMOs (the areas marked in grey and orange in Table 8). This requires combining knowledge from various disciplines of risk assessment, such as of chemicals and plant protection products, and considering the different aspects that are assessed. This can help to acquire a complete picture of the potential risks of new biotechnological applications.

The second aspect involves broadening the knowledge needed to adequately perform the risk assessment (marked in blue in Table 8). This requires bringing together and combining existing scientific and applied knowledge and information and, where necessary, generating new knowledge. For example, much knowledge and information is already available in scientific literature, such as fundamental knowledge on population dynamics in insects. Combining and integrating this knowledge with the available knowledge about gene drives, for example with the help of mathematical models, can generate useful input for the risk assessment of insects with a gene drive.

In a general sense, many initiatives are already being taken in an international context in order to develop methods and knowledge for risk assessment, such as initiatives within the OECD or regional and national initiatives. In the Netherlands, development of knowledge on safety and modern biotechnology is already taking place, and new developments in this area are closely followed. Examples include the ongoing research programme 'Biotechnology and Safety' [87], the Trend Analysis Biotechnology of COGEM and the Health Council of the Netherlands [1], publications on gene drives such as those of the RIVM [46, 76] and the research programme Ecology Regarding Genetically Modified Organisms (ERGO; 2006-2012) [81]. This policy report emphasises the importance

of continuing to proactively identify developments in modern biotechnology in a broad sense.

Due to the international nature of these developments, it is also important to make the developments and signals in the Netherlands more visible in an international context. This provides an opportunity to focus on the development of the specific knowledge and the way in which it can be obtained, also in an international context. Existing networks such as the OECD and the CBD provide a first platform.

Findings in context

The aim of this study was to evaluate the suitability of the existing GMO risk assessment for new biotechnological applications. Although this policy report addresses risk assessment only, the picture emerging from this policy report is in line with the conclusions of COGEM and the Health Council of the Netherlands in the latest Trend Analysis Biotechnology: existing regulations are no longer compatible with the field of biotechnology, with all the new applications that have recently been developed and are expected in the near future and with respect to convergent technologies [1]. For example, a number of aspects emerged during the study that clearly show the limits of existing legal frameworks:

- Due to the various legal frameworks that apply to the wide range of applications, the risk assessment methods are also classified accordingly. Moreover, this policy report describes new biotechnological applications to which multiple legal frameworks apply, such as gene therapy applications that fall under both the medical framework and the GMO framework. New biotechnological applications have also been described, such as cell-free systems, for which it is currently uncertain whether any existing legal framework applies. It is expected that more applications will emerge for which the relevant assessment framework is does not fit [1].
- For example, the application with algae in a semi-closed system described in this report is at the interface of applications under containment and environmental introduction. From the perspective of the risk assessment, this is not a problem; when assessing the risks of applications that are introduced into the environment, the degree of exposure to the environment is also taken into account. The expectation is that in the future, synthetic biology will generate more applications at this interface, such as a detection instrument with living synthesised bacteria in a sealed medical device [88]. Consequently, the strict legal separation between applications under containment and applications that are introduced into the environment is under pressure.
- Due to the increasing diversity of biotechnological techniques, there are more possibilities to obtain genetically identical organisms in different ways. For some biotechnological techniques it is unclear whether their use results in a GMO, as defined by GMO legislation.

Future biotechnological applications, such as modifications to the germline, gene drives or xenobiology – in which new forms of life are designed – impact the foundations of life and evoke ethical questions. This elicits a broad discussion about the ethical and societal aspects that these developments entail.
Sheila Jasanoff and Benjamin Hurlbut recently argued in *Nature* [89] that a coordinated international approach is desirable to ensure careful reflection on biotechnological innovations.
The lower house of parliament the Netherlands has also recently requested such a dialogue.[8] In its response[9] to the aforementioned trend analysis, the coalition government has announced that it wants to modernise its policy and regulations on the safety of biotechnology, so that the policy and regulations can keep pace with the rapid technological developments. The aim is twofold: to utilise the opportunities offered by biotechnology while ensuring the safety of people and the environment. The societal dialogue is part of this process, and the present study can help to improve that dialogue.

[8] Parliamentary Paper 27428, No. 340; the Bosma (VVD)/Van der Velde (PvdA) motion in which the coalition government is invited to “initiate a societal debate in which the public would become engaged with current developments in biotechnology”.
[9] Parliamentary Paper 27428, No. 335; Policy response to the Trend Analysis in Biotechnology 2016.

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Abbreviations and terms

Abbreviations

ASGCT	American Society of Gene and Cell Therapy
ASO	Antisense Oligonucleotide
CBD	Convention on Biological Diversity of the United Nations
CBG	Medicines Evaluation Board
CCMO	Central Committee on Research Involving Human Subjects
COGEM	Netherlands Commission on Genetic Modification
Ctgb	Board for the Authorisation of Plant Protection Products and Biocides
CRISPR/Cas9	Clustered Regulatory Interspaced Short Palindromic Repeat/CRISPR associated protein
dCas	dead Cas
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency (in the United States)
ESGCT	European Society of Gene and Cell Therapy
GM	genetically modified
GMO	genetically modified organism
IenW	Ministry of Infrastructure and Water Management
miRNA	microRNA
mRNA	messenger RNA
NAS	National Academy of Sciences (in the United States)
NIH	National Institutes of Health (in the United States)
RIVM	The National Institute for Public Health and the Environment
RNA	ribonucleic acid
RNAi	RNA interference
siRNA	small interfering RNA
TALEN	Transcription Activator-Like Effector Nuclease
VWS	Ministry of Health, Welfare and Sport
ZFN	Zinc-Finger Nuclease

Terms / definitions as used in this policy report

EpiEffector
Synthetic fusion protein consisting of a DNA recognition domain and a chromatin-modifying protein domain, which specifically changes the epigenome.

Expert judgment
The assessment of one or more experts based on his/her personal knowledge and experience.

Facilitating technologies
Technologies that play a supporting role in enabling new developments, such as those in modern biotechnology.

Green biotechnology
Agricultural biotechnology. This focuses on plant breeding and selection to obtain more productive and resistant seeds, plants and other resources.

Information
Data.

Knowledge
The ability to place information in the correct context and assess this information, as in the context of risk assessment.

Germline cells
Cells that give rise to the gametes of an organism. Changes in these cells are passed on to subsequent generations.

Minimal cell
A cell with the minimal functions needed for survival.

Modern biotechnology
Techniques involving direct intervention into the genetic material of organisms (such as animals, plants, bacteria). This is not the case with classic biotechnology.

Orthogonal system
An alternative biological (or xenobiological) system that uses functionally similar – but chemically different – molecular building blocks in the genetic code or during transcription or translation.

Other applications in biotechnology
Applications in biotechnology that cannot be unambiguously classified under red, white or green biotechnology.

Protocell
Cell-like system that contains all biological components but cannot replicate; forerunner of a living cell.

Risk assessment
A step-by-step process to evaluate the potential risks of a substance, application or product, such as potential risks to human health or the environment.

Risk assessment method
A method to perform a risk assessment that has been developed for a specific group of applications, such as GMOs, chemicals or plant protection products.

Risk assessment methodology
A general risk assessment framework that is used generically to assess risks, regardless of an application.

Risk management
Measures to control or limit potential risks.

Red biotechnology
Medical biotechnology. This focuses among other things on the production of vaccines and antibiotics, gene therapy, regenerative therapies, creation of artificial organs and new diagnostics for diseases.

Somatic cells
Body cells that are not reproductive cells.

White biotechnology
Industrial biotechnology. This focuses among other things on biocatalysis in industrial processes, for example for processing producing chemicals, materials and energy.

Appendix 1 New developments: drivers and barriers

In the various application areas of modern biotechnology, certain drivers and barriers influence the rate of development and the emergence of new applications. This can have a major impact on the time period in which new applications are expected. Therefore, the main drivers and barriers that were identified in the three exploratory studies [5-7] are briefly described here.

Red biotechnology
In medical biotechnology, we identified two developments as the main drivers. The first concerns developments in detection and screening technology. This technology can be used to detect genetic abnormalities that lead to disease or off-target effects (changes in the DNA or RNA that are not at the intended location) of gene editing techniques. The second development is that in synthetic biology. Due to this development, increasingly accurate modifications can be made in the genomes of patients, and small therapeutic molecules can be introduced into the cell in a different way. [6]

For red biotechnology, ethical aspects and patient safety are seen as the most important barriers. With regard to patient safety, two technological aspects play an important role: 1) how to prevent side effects and off-target effects and 2) how to administer the biotechnological medicine to patients so that it reaches the correct cells in the correct dose. The ethical discussions focus on changes in the DNA of germline cells (oocytes and sperm cells), whereby the treatment also affects subsequent generations. Modification of the genome in germline cells in human subjects is prohibited in the EU [90]. In the Netherlands this provision has been implemented in the Embryo Act,[10] which prohibits the deliberate modification of genetic material in the nucleus of human germline cells that result in pregnancy. [6]

Green biotechnology
There are two drivers behind developments in green biotechnology: firstly the increased efficiency and precision of modifications. The increasing speed of sequencing (reading DNA sequences) and developments in bioinformatics have contributed greatly to this development. The second driver can also be seen as a barrier. It stems from the fact that the EU has regulations against placing GM plants on the market. The development of applications, particularly those involving genome editing in the EU, will therefore depend heavily on a possible exemption of these techniques from these regulations. [7]
In plant biotechnology, methods are being developed to modify plants in a targeted way so that end product does not contain any foreign DNA. Examples are cisgenesis and intragenesis, in which only genes of the same species or of closely related species are introduced, such as disease resistance genes. Cisgenesis and intragenesis are not new techniques; they use recombinant DNA techniques. Another

[10] Embryo Act, Article 24, paragraph g. http://wetten.overheid.nl/BWBR0013797/2013-09-27

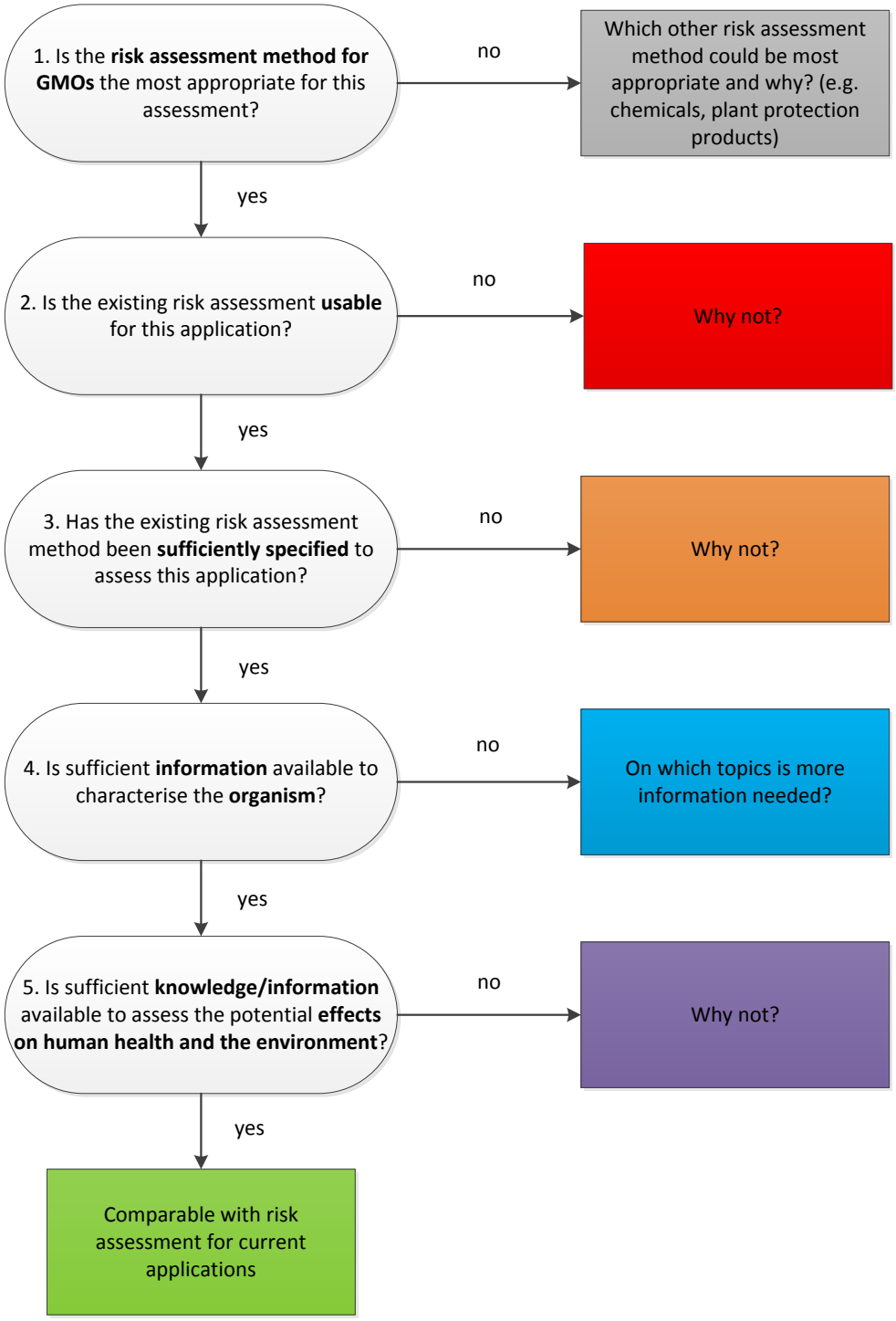
development involves creating mutations or temporarily disabling genes by inserting a recombinant DNA construct into a plasmid (a 'carrier') that is only temporarily present in the cell and then disappears. Applications include accelerated flowering in trees due to the temporary presence of the new DNA or providing herbicide tolerance. [7]

White biotechnology

The facilitating techniques described in Section 2.2 are important drivers for developments in white biotechnology. New sequencing techniques are making it easier to read and analyse many DNA sequences very rapidly. This can be used in industrial biotechnology for the development and subsequent monitoring of new strains or microorganisms. Assembling pieces of DNA (DNA synthesis) in a self-chosen sequence is also an essential basic technique. Improved technical possibilities, automation and robotisation are helping to accelerate the development of microorganisms for industrial production.

Economic considerations are the most important barriers to the use of new biotechnological techniques. In white biotechnology, the products guide the development; their production must be economically feasible. A new technique is only used when it has a clear advantage over the current technique in terms of costs or results. Although this barrier also plays a role in green and red biotechnology, it is most pronounced in white biotechnology. [5]

Appendix 2 Original question structure



Appendix 3 Overview of experts involved

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The authors of this policy report have expertise in the risk assessment of agricultural, industrial and medical biotechnology and synthetic biology.

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Reviewers
The reviewers involved in this policy report have expertise in agricultural, industrial and/or medical biotechnology, assessment of medicines and drug research, nanotechnology and bio-nanotechnology, biocides, chemicals, plant protection products, synthetic biology and/or risk assessment.

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The reviewers contributed to this policy report based on their expertise. The institutes to which they are affiliated have not been requested to approve the content of this policy report.

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