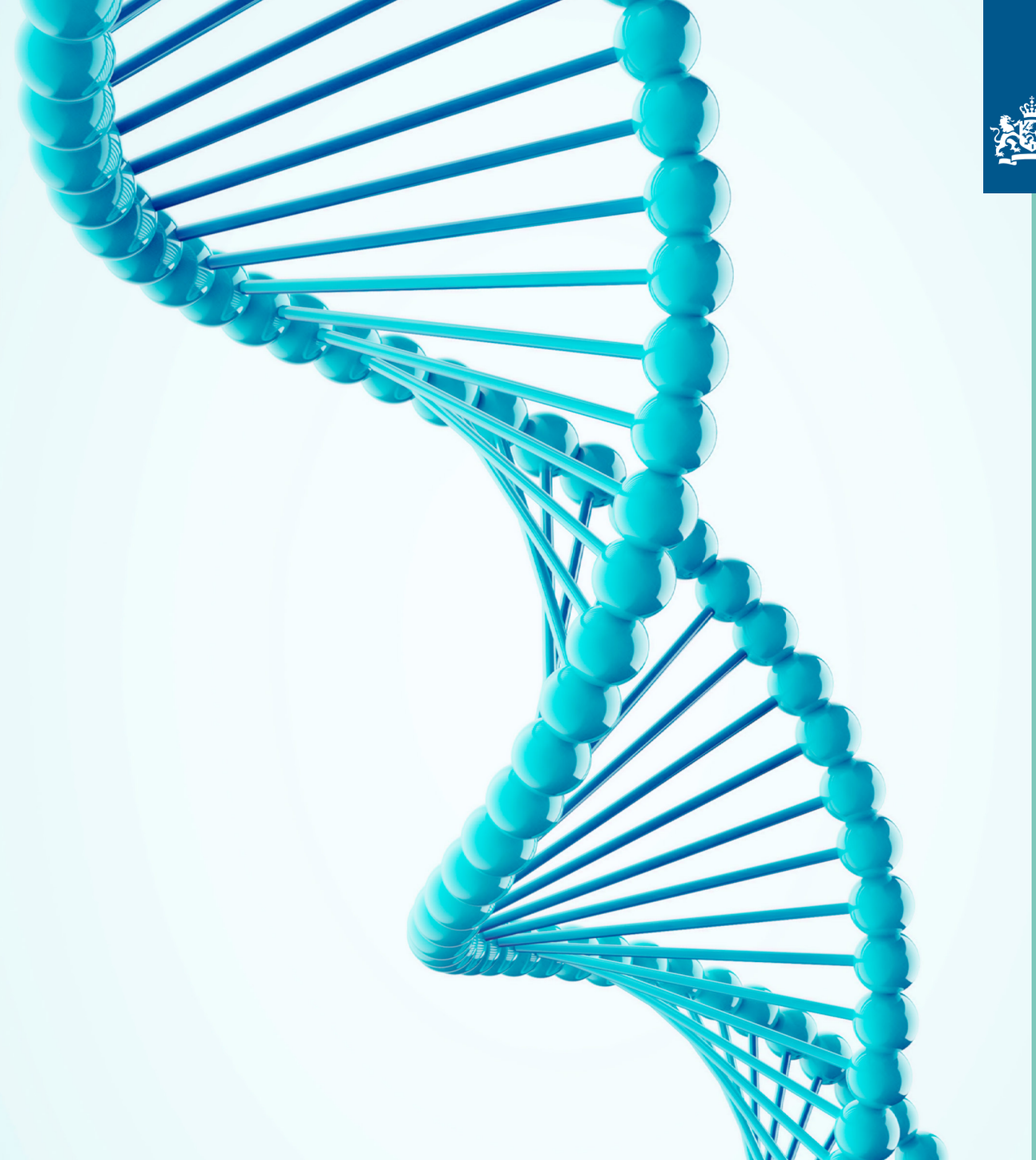


National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Safety framework Biotechnology

Basic knowledge with links to in-dept information

Preface

This material can be of interest to you, as environmental safety is important when working with biotechnology.

This document will help you understand why the environmental safety aspect of biotechnology is important, explain how you can assess safety, and give you insight into the regulations that apply to your current and future activities with biotechnology.

Target audience and learning objectives

For whom:

MSc and PhD students and researchers interested in safety biotechnology.

What is to be learned:

- Know what environmental concerns biotechnology may lead to in the lab and in the field.
- Understand the need for and logic of the environmental safety framework for biotechnology.
- Understand the principles of a risk assessment.
- Understand how in general the safety framework is translated into regulation.
- Understand that biotechnology may raise concerns beyond environmental concerns.

Each section ends with a summary of the key elements in the section.

1.

Safety of biotechnology with a focus on the environment

How potential safety concerns about applying modern biotechnology have reached the agenda

With the initial use of the recombinant DNA technique in the early 1970s, scientists realised the potential risks of the new possibilities of exchanging genetic material between species (see cartoon).

The scientists worried about the novel possibilities that the recombinant DNA technique would make possible:
- enabling non-natural exchange of genetic material between organisms;
- combining genetic material from different species.

These novel possibilities raised concerns about:
- new genetic combinations that can lead to new (traits in) organisms;
- unknown risks of the new organisms for human health and the environment.

In sum, the consequences of the novel technique had been hard to foresee.

Concerns, discussion and guidance to deal with potential hazards

1. The discussion amongst scientist during the Asilomar conference in 1973 resulted in an open letter in Science.

Gradually a public debate begun that has continued until now, although the issues that they are centered around have evolved along with the technique and its applications.

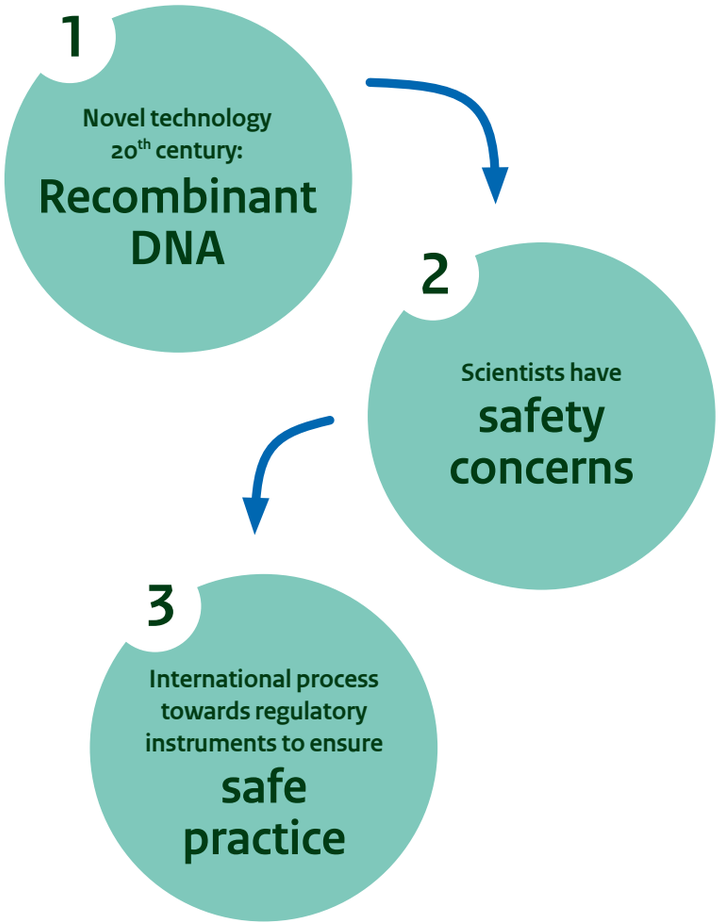
Remarkably, this situation in which scientists started the discussion is different from the situation with other environmental concerns such as chemical pollution or aircraft noise. In the latter, the public concern generally initiated the public debate.

2. The scientific discussion and concern resulted in guidelines issued by the National Institutes of Health (NHS) in the USA.

Globally this has resulted in safety guidelines of the Organisation for Economic Co-operation and Development (OECD).

The early guidelines provided the basis for the legal framework that came into force in Europe in 1990.

The process from concern to regulating environmental safety when applying biotechnology



Concerns translated into protection goals

The awareness of concerns called for insight in the potential adverse effects of activities involving modern biotechnology and criteria for assessment. The latter have given rise to establishing protection goals: environmental goals and health goals that should not be jeopardized by applying biotechnology. The health goals considered here refer to general human health effects. Human health of workers and patients is not addressed here, as this is dealt with in designated safety frameworks.

The environmental protection goals that are determined for using biotechnology are:

- biodiversity conservation;
- protection of species (plants, animals);
- conservation of ecosystems.

More insight in potential adverse effects

In the course of time, a range of potential adverse effects have been identified that could jeopardize one or more protection goals. To give you an idea what these effects are, look at the following list:

Genetic modification could have effects for:
- the pathogenicity of the organism;
- the dispersal and survival of the organism in the environment;
- invasiveness and persistence;
- selective advantage, disadvantage;
- gene transfer;
- target effects;
- non-target effects;
- effects on human health;
- effects on animal health;
- effects on biogeochemical cycling.

What environmental safety do we aim for?

Defining safe use of biotechnology

Looking at the potential adverse effects and the protection goals, the question arises what is to be considered as safe use of biotechnology. Think back to the public concern and the caution of the scientists that we mentioned earlier. In policy in the Netherlands, these concerns have been translated into the following definition of safety in relation to biotechnology: Only when activities involving biotechnology have *a negligible risk*, are these activities considered as being safe.

The term ‘negligible risk’ calls for further explanation.

Negligible risk

Ideally, policy in the Netherlands would like to define safe activities with biotechnology as activities with biotechnology that have no risk at all or zero risk. Zero risk, however, is not feasible. Therefore, zero risk has been operationalized into negligible risk, meaning that the potential adverse effects of activities with biotechnology are estimated to be so small that these can be considered negligible.

Reaching negligible risk: a key element

Most of the adverse effects will materialize only when the genetically modified organism (GMO) is dispersed into the environment.

Look at the following examples of dispersal. Working at a lab bench may cause a dispersal of micro-organisms by aerosols. Adverse effects can subsequently result.

Growing genetically modified (gm) plants in the field may result in dispersal of gm pollen and/or seeds that might cause potential adverse effects.

Succeeding in preventing dispersal of the GMO by taking appropriate measures is therefore regarded as essential for the attainment of negligible risk.

Photo by Guy Akkermans, DuRPh project, Wageningen University & Research

Preventing risk...

Relevant policy has developed the following line of thinking to achieve negligible risk when activities with biotechnology take place in a contained environment, e.g. in an installation or a laboratory:

- Risk management measures adequate to the risk occurrence should be taken (**see page 15 to 17 in module 2** ▶).
- When the activities take place in a contained environment and risk management measures are taken, adverse effects can take place only due to an accident and/or unintentional release.

So what to do when contained activities are not feasible, e.g. when the aim is to grow a gm crop?

Policy developed a step-by-step approach, that starts with containment. The experimental activities can be taken a step further ahead after the previous step has been proven safe. Then the next step under less restricted experimental conditions can take place. By providing evidence of safe use in each step, the activities are allowed to be taken a step further and so forth (**see page 24 in module 3** ▶).

Summary Safety and Environmental Protection

- In the area of biotechnology, scientists started the discussion on working safely with biotechnology. Subsequently, a public discussion started in relation to the concerns.
- Concerns are related to human health and the environment.
- Concerns addressed as a set of adverse effects are related to the protection goals.
- Activities with biotechnology are considered to be safe when there is a negligible risk.
- Containment is a first step in achieving negligible risk.

2.

Addressing the risks from biotechnology: from adverse effect to negligible risk

A need for risk definition

As a researcher, besides knowing what kind of adverse effects may arise from your experiments, you also need to know what the likelihood is that they may occur. Then you know what the risk occurrence is.

This section will elaborate on the method to determine the possible risks and the measures that can be taken in order to achieve negligible risks.

When talking about risks there is a general risk formula that is used in any technology area:

Risk = hazard x frequency

The formula points out that risk differs from hazard, but what does this mean?

Understanding the difference between risk and hazard

Hazard is the possibility of something causing harm. In the picture you see a flask on a table with a toxin producing bacterium culture. The toxin is the hazard and may cause harm to human health and the environment.

Risk refers to the probability of harm occurring. Applied to the picture, this means that if the flask is at the edge of the table, the probability that someone may bump against the flask causing it to break and releasing the toxin (causing harm) is high. In this case, risk is the probability that the toxin is released from the flask.

To minimize the risk, action is needed.
For further understanding the difference between risk and hazard click here.

The picture above illustrates that risk and hazard have a different meaning.

Risk definition in biotechnology

In biotechnology, the risk formula makes use of a different terminology: Hazard is named adverse effect. Frequency is named likelihood

This results in the formula:

Risk = adverse effect x likelihood

The risk formulation using the elements ‘adverse effect' and ‘likelihood’ is defined in the GMO regulation.

For determining the risks of biotechnology, the formula is not used in a numerical way, but in a qualitative way. This means that adverse effect and likelihood have to be identified and substantiated based on all available scientific knowledge.

Some notes on adverse effect and likelihood

Adverse effect

In the previous module you have learned what kind of effects are recognized as adverse effects resulting from biotechnology. In the risk assessment, each effect has to be considered whether it may occur or not during the activity.

Furthermore, an adverse effect like pathogenicity may differ in its level of effect. For example, one pathogen may cause a cold from which a person can easily recover, whereas another pathogen may cause a deadly infection. The latter is of course a much more serious effect. As a consequence, **an adverse effect** has to be considered **in combination with its severity.**

Adverse effects from a lab setting may occur due to an incident, an unintentional release of the GMO from the lab into the surrounding environment.

Likelihood

Having identified a potential adverse effect, the risk then depends on the possibility that the adverse effect might actually occur, the **likelihood that an effect may occur.**

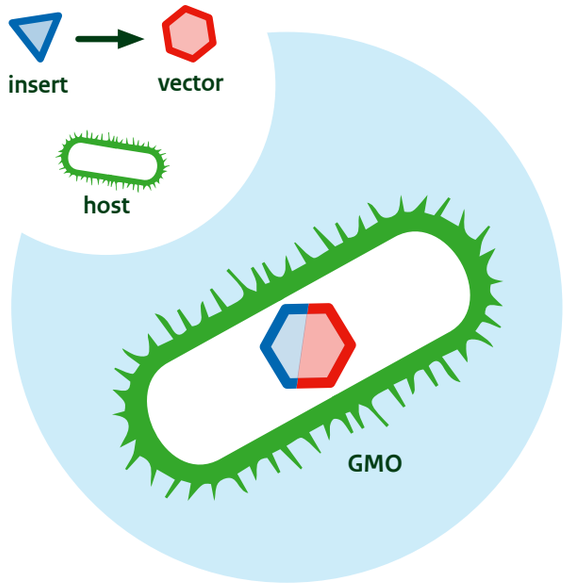
As we aim in biotechnology for negligible risks, the risk assessment process is to be followed by risk management measures that will minimize the risks to negligible risks. We will first explain the steps in general, and then the steps will be illustrated.

Risk assessment and risk management: general steps

- **Step 1:** identify the potential adverse effects and their severity.
- **Step 2:** determine the likelihood that an effect may occur.
- **Step 3:** identify the risk level by combining step 1 and 2.
- **Step 4:** determine the appropriate risk management.
- **Step 5:** determine overall risk for human health and the environment, which needs to result in a negligible risk.

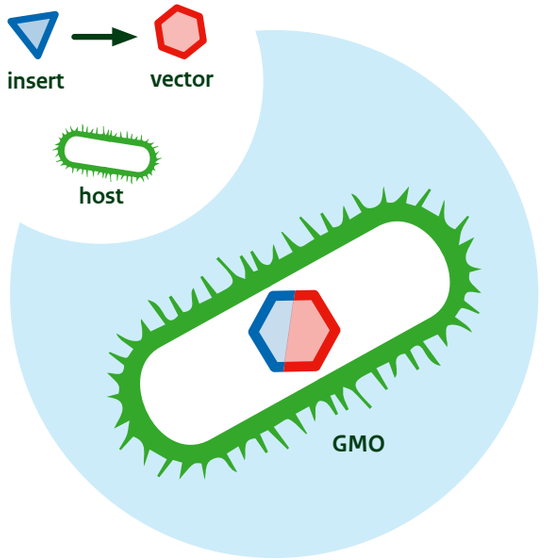
To illustrate the steps, we apply the steps to a biotechnology activity with a genetically modified organism (GMO).

A GMO is being composed from a wildtype organism (the host) that is genetically modified with a vector carrying an insert:



Step 1: Identification of adverse effects and their severity

Identification of the adverse effects starts with looking at the adverse effects of the individual elements the GMO is composed of. The figure shows what is meant by the elements: it is the host (the organism that is to be modified) and the vector carrying an insert. The combination of the elements host – vector – insert result in the GMO. The adverse effects of the resulting GMO need to be considered as well.



As the severity of an adverse effect has to be considered as well, the range of the severity component for the adverse effect of 'pathogenicity' of the GMO and its constituent elements is given in brackets:
a. the host (mild or strong pathogenicity);
b. the insert including the vector (a housekeeping gene or a toxin encoding gene); and
c. the resulting GMO: the interaction between a and b needs to be considered (the toxin-encoding gene is not expressed versus high expression in the GMO).

Identification of an adverse effect and the severity are based on a scientific rationale. Each adverse effect identified must be considered even if its severity is uncertain.

Step 2: Determine the likelihood

The likelihood that an adverse effect may occur is estimated by taking into account the characteristics of the activity and the surrounding environment.
The likelihood that the flask containing a GMO culture gets broken is real. Nevertheless, this likelihood must be determined in terms of negligible, low, medium or high.

Step 3: Identify the risk level

The level of risk that may result from an adverse effect is identified in this step. The diagram helps to determine the risk level of the particular activity you are assessing. In this diagram, the level of severity of the adverse effect is presented horizontally and the level of likelihood vertically.

By crossing the horizontal and vertical axis, their crossing shows the risk level and resulting risk class for the particular GMO activity.

The risk level and class of activities with a GMO

		Severity of potential adverse effects			
		Negligible	Low	Medium	High
Likelihood of occurence of potential adverse effects	High	Low **Risk class 2**	Medium **Risk class 3**	High **Risk class 4**	High **Risk class 4**
	Medium	Low **Risk class 2**	Low **Risk class 2**	Medium **Risk class 3**	High **Risk class 4**
	Low	Very low **Risk class 1**	Low **Risk class 2**	Low **Risk class 2**	Medium **Risk class 3**
	Negligible	Very low **Risk class 1**	Very low **Risk class 1**	Low **Risk class 2**	Low **Risk class 2**

Step 4: Determine the appropriate risk management

The outcome of step 3 is a risk class to be assigned to the activity. The risk class determines what set of measures are needed to manage the risks and aim for negligible risk.

For activities in the laboratory, the risk class corresponds with the risk management measures of the corresponding biosafety level (BSL) of the required lab: an activity of risk class 1 may take place in a BSL-1 laboratory, that of risk class 2 in a BSL-2 laboratory, and similarly for BSL-3 and BSL-4.

Risk management measures are:
- rules that determine the working practice, and
- physical measures.

Together, the risk management measures determine the level of containment.

It will be clear that an activity with either an adverse effect that is more severe and/or a higher likelihood of occurrence, requires more stringent risk management measures. The activity then needs a higher level of containment, e.g. a BSL-3 instead of BSL-2.

The following pictures show the different BSL laboratories and some measures.

Risk class 1: BSL-1 risk management measures

A BSL-1 laboratory is the suitable containment for working with apathogenic microorganisms, e.g. labstrain *Escherichia coli* K12.

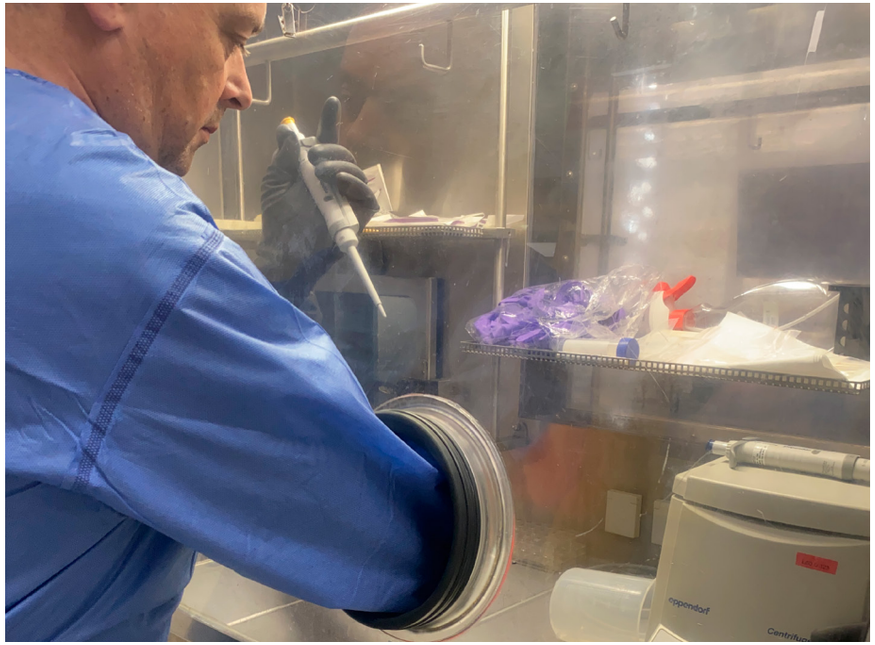
The severity level of the adverse effect pathogenicity is relatively low. As a result, the risk management measures can be limited in order to obtain a negligible risk for the environment.

The activity is therefore allowed at a working bench.

Risk class 2: BSL-2 risk management measures

A BSL-2 laboratory is the suitable containment for low pathogenic microorganisms that are unlikely to spread and for which an effective treatment is available, e.g. the adenovirus, a virus that causes a cold.

The most important risk management measures in a BSL-2 laboratory are:
- aerosol forming activity has to be performed in a safety cabinet of class II (see picture);
- access to the lab is restricted to trained personnel.



Risk class 3: BSL-3 risk management measures

A BSL-3 laboratory is the suitable containment for working with seriously pathogenic microorganisms that are likely to spread and for which an effective treatment is available, e.g. *Mycobacterium tuberculosis* causing pneumonia.

Risk management measures in BSL-3 specifically prevent the release of the microorganism by aerosols from the laboratory into the environment. This is achieved, for example, by the presence of an airlock, by negative air pressure in the laboratory, and/or by working in a biosafety cabinet of class II or III (see picture).

Risk class 4: BSL-4 risk management measures

A BSL-4 laboratory is the suitable containment for working with highly pathogenic microorganisms that are likely to spread, and for which no effective treatment is available, e.g. Ebola virus causing hemorrhage frequently resulting in death.

In addition to the risk management measures of BSL-3, measures are taken to prevent any contamination of the worker in the laboratory with the microorganism.

Examples of additional containments

On the previous pages, containment measures (BSL-1 to 4) are shown for GMO activities in a laboratory working with microorganisms. There are also specific containments for activities with GM plants and animals or for activities on an industrial scale. Here are some examples.

Greenhouse: *measures to prevent dispersal of plant parts into the environment.*

Industrial bioreactor: *measures to prevent a large spill into the environment.*

Animal facility: *measures to prevent escape of the animal.*

Step 5: determine the overall risk

The last step in the process has to ensure that the overall risk of the activity is indeed negligible. In this step, you check whether the risk class and the containment measures as determined in step 4 are adequate to obtain an overall risk of the GMO activity that leads to a negligible risk for human health and the environment.

- If negligible risk is not reached, you have to think about what additional measures are needed.
- Or you could consider whether some containment measures may be too stringent In these cases, steps 1 to 4 have to be reiterated.

Point of attention

The method we have described here, implies that the determination of risk is based on a *worst case scenario*. If an adverse effect or likelihood is uncertain, this also has be taken into account. This principle of worst case is based on precaution.

Summary Addressing the risk from biotechnology

- Risk and hazard are different concepts.
- In biotechnology, the elements of the general formula for determining risk are named adverse effect (in relation to its severity) and likelihood.
- The process to achieve negligible risk takes five steps, from determining the risks to taking measures to achieve a situation with negligible risk.
- The outcome of the assessment results in risk management measures.
- Risk management measures are adequate if they reduce the risk to a negligible risk.
- Adverse effects, risk assessment and containment measures are all laid down in the GMO regulation.

3.

GMO regulation

The genetically modified organism (GMO) regulation

European and Dutch GMO regulations provide the safety framework for activities with GMOs in the Netherlands.

This regulatory framework aims at a negligible risk of the activities with GMOs for human health and the environment.
The method of risk assessment of GMOs that was elaborated in the previous chapter, as well as safety measures and administrative procedures for permitting activities, are stipulated in this regulation.

Aim of the GMO regulation:
To assure a negligible risk to human healt and the environment due to GMO activities.

Central elements in the GMO regulation are:
- the GMO and its characteristics;
- the nature of the activities that take place.

This module discusses the GMO regulation and additional regulations that may apply to GMOs.

What is regulated?

The scope of the GMO regulation, i.e. what is and what is not regulated, is important. Thus, the definition of a GMO is key.

According to the European GMO legislation the definition of a GMO is as follows:
A genetically modified organism (GMO) is an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.

This means that organisms that meet the criteria of this legal definition are subject to the GMO legislation.

Note that humans are also organisms and could (in the future) be modified as well. Humans however are exempt from this legal definition and consequently from the GMO regulation.

Different kinds of GMO activities

As the risk of adverse effects depends on the kind of activities, the regulation makes a distinction between two kinds of **activities** with GMOs:

1. **Contained use:** Activities with GMOs that are carried out in a contained space, for example in a laboratory.
2. **Deliberate release:** Activities with GMOs that involve release in the environment.
   A further distinction is made between:
   - Introduction into the environment (small scale);
   - Placing on the market.

The releases that takes place with an introduction into the environment are relatively small and are usually limited to certain areas. Examples are a **field trial** with gm plants and a clinical trial with a gm medicine.

Placing on the market is a release on a large scale, i.e. when GMOs are sold as a **product.** For each category of activities, the regulation prescribes the necessary regulatory requirements.

Contained use

With contained use, the GMO is handled in a contained space. The risk management measures to ensure safety are related to the laboratory or installation and working practice.

Basically, when activities with GMOs take place in contained use, the GMO is not released into the environment. However, this could happen unintentionally by accident.

The GMO regulation prescribes the appropriate **containment measures** that must be taken, to assure the prevention of an unintentional release into the environment of the GMO.

The risks of the specific GMO determine what regulatory procedure must be followed in order to allow for the intended activities. A notification or a permit can be needed.

Photo by Guy Akkermans, DuRPh project, Wageningen University & Research

Deliberate release: introduction into the environment

As indicated before, introduction into the environment refers to the release of a GMO into the environment, on a small scale. Notice that prior to this step, the GMO has been developed and tested in a contained space under the 'contained use' regulation (**see page 25** ▶). To test how the GMO behaves outside the lab, a GMO can be released into the environment on a small scale, e.g. in a field trial or a clinical trial.

For all activities, an approval is needed and the procedure also includes a public consultation round.

Again, the intended activities involving introduction into the environment are only allowed if the risks are negligible. To ascertain this, the regulation requires a risk assessment.

To achieve negligible risk, adverse effects of a GMO can be minimized by, for example, using an organism that cannot reproduce. By taking appropriate risk management measures, the risks can be further diminished to 'negligible'. Examples of risk management measures are: preventing contact with wild relatives or preventing spread of the GMO in the environment.

Deliberate release: Placing on the market

Placing on the market refers to the situation that a GMO is sold as a product (on a large scale). Notice that prior to this step, the safety of this GMO has been proven in small scale introductions into the environment. Under these circumstances, the safety data required for an application for placing on the market are collected.

Placing on the market requires an EU approval. If the EU legal procedure leads to a market approval, the EU approval applies to all the countries in the EU.

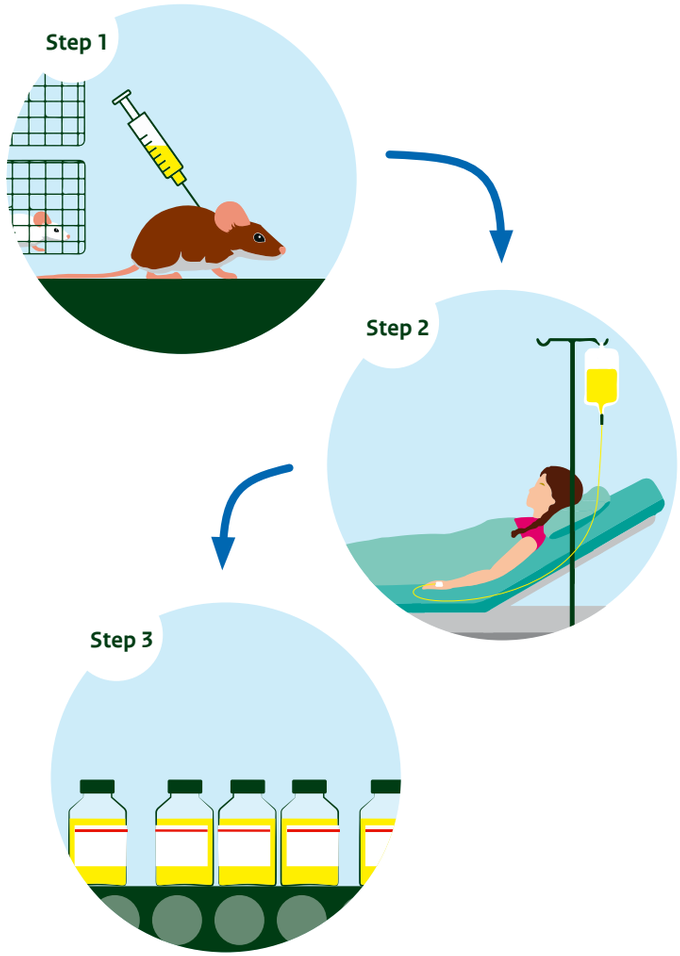
For a market introduction, labelling and monitoring of the GMO are required in order to detect possible unexpected effects on the environment. For more reading on labeling, see: GMO Labeling Laws per Country - Global Food Safety Resource. The necessity for monitoring is laid down in the GMO regulation. For labelling, a European order is in force (**see page 25** ▶).

Overview of step-wise introduction

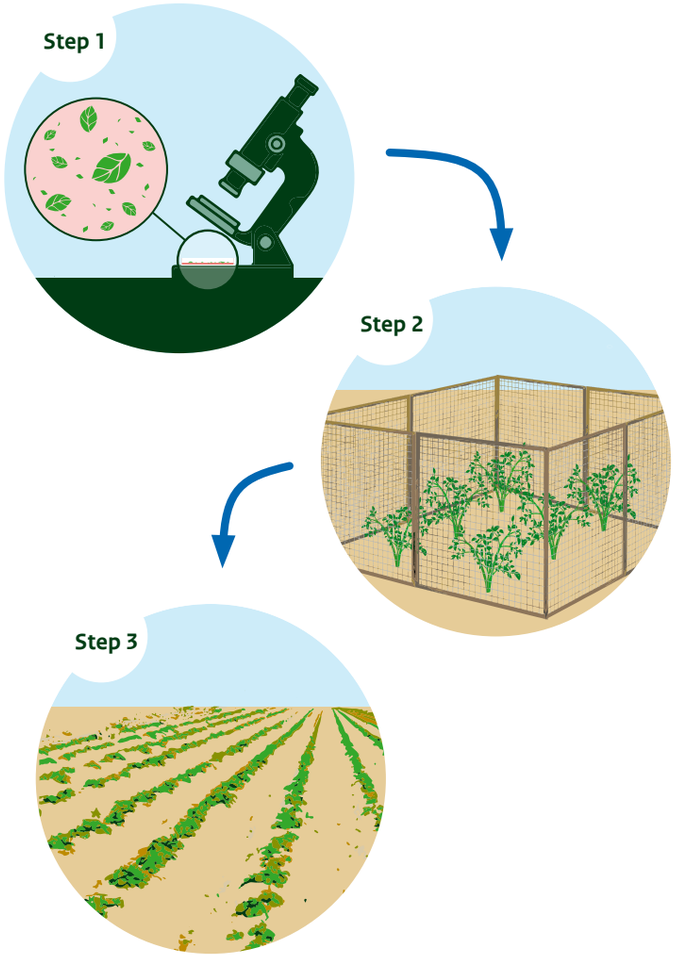
The development of a new GMO towards commercial release is a step-wise process. With every step, the exposure to the environment increases.

Step 1: Contained use
Step 2: Small scale release
Step 3: Placing on the market

Medical application

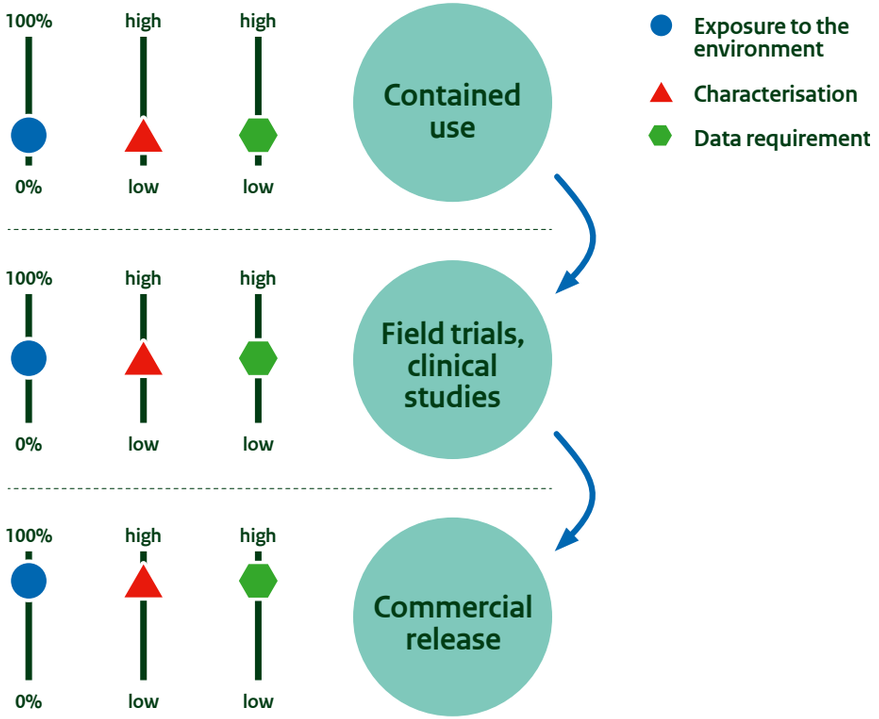


Agricultural application



A stepwise introduction: the legal requirements

In the development of a GMO, the knowledge about the GMO and its possible adverse effects increases. This is meant by characterisation in the figure below. By doing experiments, more data can be collected to further confirm this characterisation. A GMO that has proven to be safe in a first step can be allowed for a next step with an increase in exposure to the environment. At the last step, the commercial release, many safety data are (legally) required and need to be available.



GMO legislation in Europe

The safety framework for GMOs in Europe has been laid down in two Directives. A Directive is a legally binding instrument that has to be implemented in the laws of the member states of the European Union. A Directive cannot simply be used as is. Member states are obliged to lay down the rules of the Directive in their national laws. There is some room for member states to give their own interpretation, which explains why GMO regulations may differ among member states.

The two Directives are:
- Directive 2009/41/EC: Contained use of GMOs
- Directive 2001/18/EC: Deliberate release in the environment of GMOs

Important European Regulations concerning GMOs are:
- Regulation 1829/2003/EC: on food and feed
- Regulation 726/2004/EC: on medicinal products

GMO legislation in The Netherlands

Both European Directives are implemented in the Dutch regulation in the GMO decree: ‘Het Besluit genetisch gemodificeerde organismen 2013’. In the decree, the procedures are described that have to be followed before being allowed to perform activities with GMOs.

The technical details of the rules regarding the GMOs and the different activities are described in the GMO order: ‘Regeling genetisch gemodificeerde organismen 2013’.

- GMO decree: Besluit genetisch gemodificeerde organismen
- GMO order: Regeling genetisch gemodificeerde organismen milieubeheer

The purpose of the rules in this decree and order is to ensure environmental safety. Other aspects such as worker protection or ethical considerations are not part of this legislation.

Additional legislation that applies to GMOs

Safe handling of GMOs is not limited to environmental safety alone. It may also include safety aspects related to spatial planning, transport or animal testing. Depending on the specific GMO activity, these aspects can also apply to the GMO activity and additional regulation will then need to be considered.

A list (although not complete) of some of the additional regulations that also apply to a GMO activity is presented here.

- Spatial planning and the environment (Wabo, since 2022: omgevingswet)
- Transport (ADR and Wet vervoer gevaarlijke stoffen)
- Worker protection ('Arbo' / arbeidsomstandigheden besluit hoofdstuk 4)
- Waste (Landelijk afval beheersplan (LAP) sector 19)
- Disinfection (Wet gewasbeschermingsmiddelen en biociden)
- Animal testing (Wet op de dierproeven)
- Plant pathogens and quarantine (Europese Plantgezondheidsverordening (EU) 2016/2031)

Supervising the safety of GMO activities

It is important not only to impose rules but also to check if the rules are being complied with. This check is carried out on two different levels.

First, compliance with the rules is controlled by the government by means of enforcement by the Human Environment and Transport Inspectorate.

Second, every organisation that handles GMOs has to appoint a biosafety officer. This biosafety officer has to be accredited by the government and has the task of supervising and enforcing the safety of the activities within the organization independently.

For more information about the biosafety officer (BVF) see this website.

Summary GMO regulation

- GMOs are regulated by two European Directives, one for contained use and one for deliberate release.
- Both Directives are implemented in national legislation.
- The European and Dutch rules consist of procedures and measures to ensure negligible risk.
- For deliberate release, there is a stepwise introduction.
- There is supervision by the government and, at the level of the permit holder, by a biosafety officer.

4.

New developments challenge safety

New developments in biotechnology

As in any scientific field, there are constantly new developments in biotechnology. Especially in the last decade, some groundbreaking developments have taken place in synthetic biology and in new biotechnology techniques such as the use of CRISPR-Cas. These developments have had a similar impact on the discussions about safety as the recombinant DNA technique did in the 20th century.

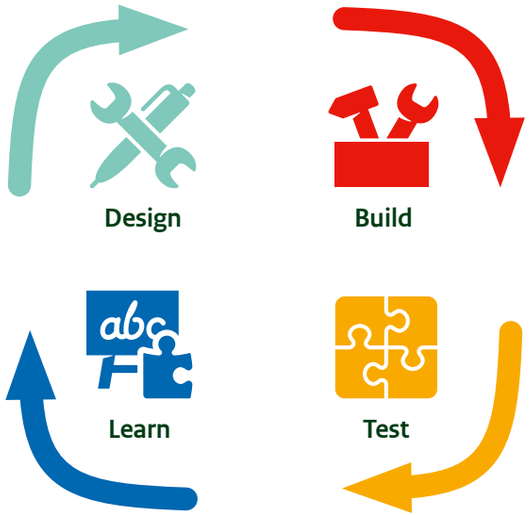
This final module discusses two developments (**synthetic biology and CRISPR technology**) that challenge the current safety regulations. We highlight some of the important concerns and safety questions. Furthermore, although the process of how to deal with the uncertainties of these developments is still ongoing, we illustrate the steps that can be taken to manage safety when there is still much uncertainty.

Development 1: Synthetic Biology (SynBio)

SynBio is defined by a European working group as the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms (see this report).

The authors of this report identified different areas of SynBio such as:
- synthetic genomes built by rewriting and synthesizing the DNA sequence;
- metabolic pathway engineering, enabling the production of new compounds by using new combinations of genes;
- xenobiology, referring to the use of non-natural compounds for DNA or proteins.

SynBio can also be regarded as a new mindset for biotechnology making use of the engineering principles: Design, Build, Test and Learn (DBTL-cycle, see Figure). As an example, a new genetic pathway can be **designed** on the computer, **built** by synthesizing the necessary DNA sequences, **testing** the pathway in a production organism, and **learning** from data whether optimalization is needed.



Safety concerns and challenges of SynBio

With the rise of SynBio, safety concerns became more complex and risk assessment more challenging due to:
- the increasing number of genetic modifications;
- the increased speed of modifications by the new technologies for DNA synthesis and genome editing;
- the use of non-standard biochemical systems in living cells.

At the same time, new safety locks such as auxotrophy and kill switches are being developed. Using a safety lock in a GMO may prevent its survival in the environment. For further reading on safety locks and their potential to prevent risks, check this report.

In their second report, the European working group recommended revisiting the risk assessment methodology for SynBio at regular intervals to ensure that risk assessment methods advance in parallel with SynBio advances.

Example of synthetic biology: orthogonal DNA

The use of non-standard DNA, so-called orthogonal DNA, is mentioned as a new area of synthetic biology: xenobiology. The use of orthogonal DNA enables the design of new genes and the encoding of new proteins.

While this development is in its early stage, an unintentional release of orthogonal DNA containing microorganisms from the laboratory raises questions concerning environmental safety. Adverse effects such as interaction of these organisms with wildtype organisms in the environment and survival as well as the possibility of horizontal gene transfer need to be assessed and may require data on e.g. survival that are not (yet) available.

Environmental safety is however not the only concern associated with this development. Ethical considerations such as the creation of new organisms, unknown in nature, and biosecurity issues such as the design of new toxins can also be raised.

Development 2: CRISPR technology

CRISPR turned out to be a groundbreaking discovery. It was discovered as being a defence system of bacteria against viruses and developed within a few years into the leading genome editing tool.

Watch this video or this video to understand what CRISPR is and how it works.

Genome editing via CRISPR enables genetic modification with high precision and speed: precision as the genetic location can be defined beforehand and speed as less individuals and/or generations are needed to obtain the desired genetic line.

There are already many crops available with a genome genetically edited by CRISPR, for example the non-browning mushrooms. Interestingly, these mushrooms are GMOs according to EU regulations, whereas they are considered as non-GMOs and approved for the market in the US.

In the following, we address gene drives as an example of a specific use of CRISPR. Gene drive technology has raised new questions that are also beyond the usual safety concerns.

Example of CRISPR technology: gene drives

A gene drive is a genetic trait that is inherited to all of the progeny. This is in contrast to the "normal" Mendelian inheritance in which only 50% of the progeny receives the genetic trait. A gene drive is therefore a very useful tool for introducing a new genetic trait in a population. This video explains what a gene drive is.

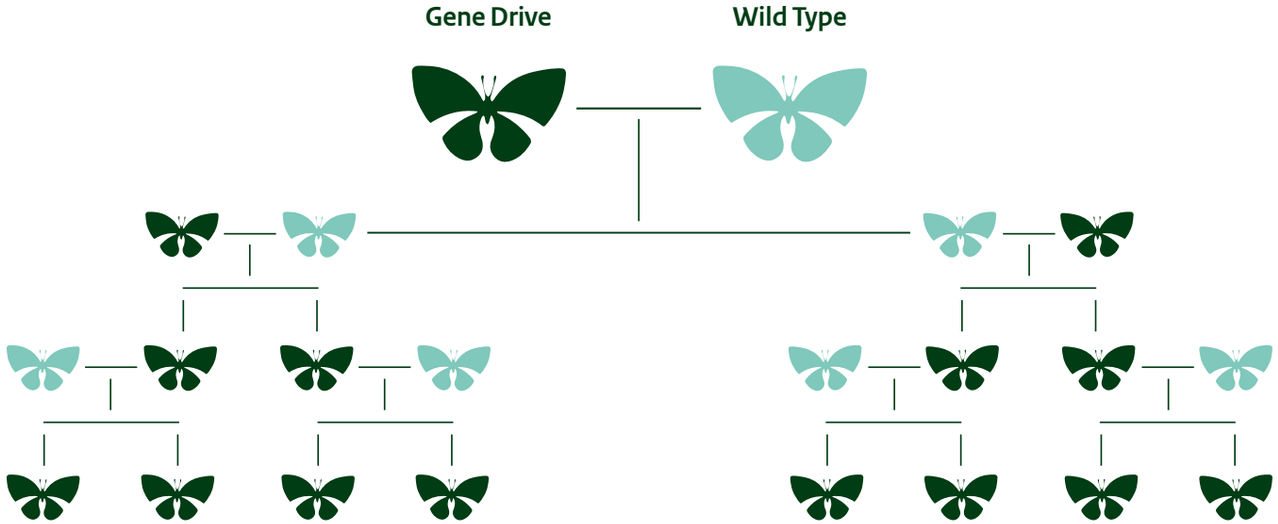
The CRISPR technology enables the development of a gene drive with (a theoretically) 100% efficiency. Next to promising applications such as malaria control, scientists immediately pointed out concerns: a **single** gene drive organism escaped or released into a local population could now potentially change every organism of that population in the world.

A US group of scientists published their concerns in a 'Science paper': Regulating gene drives. This paper not only addresses environmental concerns but also points out gaps in the regulation.

Gene drive: regulating the environmental concerns

After the first announcements on creating CRISPR gene drives, RIVM reported the shortcoming concerning gene drives in the GMO regulation to the Dutch ministry (see this report). At that time, the Dutch GMO regulation was organized in such a way that an organism genetically modified with a gene drive could be handled at a level that is comparable to BSL-1. This level of containment is clearly insufficient to provide the proper containment to an organism carrying a gene drive.
As there was no experience yet with the appropriate containment measures for gene drive organisms, the minister decided that activities with a gene drive must be assessed by the regulator and that a permit is required for being allowed to perform such an activity.

This change in the procedure was addressed by an amendment of the Dutch regulation in 2016. In this report, the risk assessment and legal procedure for allowing activities with a gene drive in **contained use** is explained.
The The European Food Safety Authority (EFSA) has recently published their advice on the assessment of environmental risks of gene drives: EFSA advises on risk assessment of engineered gene drives.



Summary
New developments challenge safety

- New developments in biotechnology raise new safety questions and additional concerns that are not always covered by current regulation and governance.
- Just as in the 1970s, scientists raised their concerns on gene drives and the topic was subsequently taken up by policy makers. As a consequence, regulations have been updated in the Netherlands.
- The environmental, social, and ethical concerns and questions raised by the new developments are more intertwined than ever.

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