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

Educational material for addressing Safe-by-Design in biotechnology:

Background information and guidance on using case material

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1. Introduction to the educational material

This document presents the background information and guidance on using the case material. The case material is presented in the form of research project examples that can be used to help address a range of safety issues, and apply Safe-by-Design in the field of biotechnology. The cases are intended to be used to stimulate group discussions. Each case is comprised of background information to the case and suggestions for group discussions.

Safety is an important aspect when working with biotechnology. When we use the term biotechnology, we refer to modern biotechnology techniques such as genetic modification and synthetic biology. Applying Safe-by-Design means incorporating safety right from the start of the development of a material, product or process and ensuring safe innovation.

The aim of providing this educational material is to let you explore the safety aspects and appreciate what Safe-by-Design (SbD) means when applying biotechnology. Using the cases involves exploring an array of tangible safety issues and becoming aware of the stakeholders involved and their responsibilities.

The educational material is comprised of:

- This document, which contains background information about the cases, the basic assumptions used to underpin the material, a general outline of the cases, and an overview of the content of the cases. An appendix has been added to provide additional information on safety and Safe-by-Design.

- Five separate cases, each consisting of: general information about the case, case descriptions and background information, and suggestions to stimulate group discussion. The cases can be downloaded and used separately.

2. Background and Overview

2.1. The educational material: underpinning assumptions

Safe-by-Design
We consider Safe-by-Design (SbD) to be a helpful approach that challenges us to adopt a fresh view of safety, bringing safety 'to a new level'. SbD refers to a comprehensive safety approach in which all kinds of safety perspectives are considered early on in the innovation process, and all the way through: from development to production, to use, recycling and waste. During this process, relevant stakeholders are involved to ensure that their input on the safety issues at hand are fully considered.
SbD demands an interdisciplinary and cooperative way of working so that all the different levels and/or perspectives on safety can be included. We need to foresee and scrutinise all kinds of safety aspects, especially in a context where (major) innovations are rapidly taking place, so that these innovations can contribute in a responsible way to society.

Safety
Safety encompasses questions regarding hazards, risks and risk reduction measures. The risk that a hazard may occur depends on the probability that the hazard may occur. See the appendix for further background information.

Addressing SbD
By addressing SbD, we expect students and researchers in the life sciences to develop a broader perspective, not only about safety but also about the socio-economic and societal factors impacting modern biotechnology. This means that you need to be aware of, and can reflect upon, the specific hazards and risks in a biotechnical research project, and develop an understanding of the measures which need to be taken, or devised, to ensure safety. Furthermore, you should be able to broaden the safety horizons with perspectives on stakeholders, the different roles and responsibilities in safety, and other important factors (e.g. socio-economic and societal factors).
The educational material is primarily focused on cognitive learning but some suggestions are also given to enrich the learning experience by providing affective learning experiences. For example by integrating the case material into role-play exercises.

2.2. The educational material and the learning experience

This educational material presents five cases, each with a different biotechnological innovation that encourages the student or researcher to learn to identify the particular hazards, risks and risk reduction measures, but also provides a starting point for an exploration of the broader safety issues.

The learning experience is provided as a group discussion of the particular case. To lead the discussion, questions are proposed that focus on the biotechnological technique itself and its consequences. This perspective originates from the safety framework established in the field of biotechnology (domain-specific safety) and is closely linked to the regulatory framework. The perspective is then broadened by questions involving e.g. stakeholders, their perceptions and concerns, and the socio-economic and societal factors. These questions refer to different levels of safety, to perspectives about safety, or to systems of influence which affect safety. Examples are: how to weigh financial costs against safety gains or how to deal with the inequalities such as those that exist between those who are burdened with the risks and those who receive the gains. Questions like these are also acknowledged in the Responsible Research and Innovation (RRI) framework[1].

General Information on the use of the cases

Knowledge area: Safety, biotechnology

Application: Either in a minor or major Life Sciences programme or within a research group interested in broadening their views on safety. The choice of cases can be adapted to the level of the programme or your field of interest.

Use and objectives: Exploring Safe-by-Design when using modern biotechnology, resulting in:

a. An understanding of the key questions related to hazards, risks and risk reduction measures (domain-specific safety) and how the safety horizon can be expanded by identifying more relevant questions.
b. Acknowledgement and understanding of:
   - How safety concerns can occur during each and every phase of the chain from development to production, application, recycling and waste.
   - The stakeholders involved and their responsibilities and influence to ensure safety along this chain.
   - The importance of this approach to research.

Preferred method: Group discussion

Time needed: See Table 1. A time window of 60 minutes per case is suggested. Depending on the level of existing knowledge on safety; the number of questions addressed and the thoroughness of the discussion you aim for, time can be shortened or extended.

Prior knowledge: 1) Knowledge of biotechnology (minor level). 2) Awareness of biotechnology regulations.

Case specific: See the case descriptions

Assignment: In general consists of: a) an introduction to safety and the Safe-by-Design approach to acting responsibly as a researcher; b) an introduction to the case(s); c) a group discussion; d) a wrap up to summarise the key notions of the discussion.

[1] E.g. https://www.nwo.nl/en/research-and-results/programmes/responsible+innovation; https://ec.europa.eu/programmes/horizon2020/en/h2020-section/responsible-research-innovation;

2.3 Structure of the case material

The case material follows the format presented in Table 1.

Table 1. Format of the case material.

Format of case material

1. General information on:
   - Biotechnology domain and focus;
   - Learning objectives;
   - Case-specific knowledge required.

2. Case description:
   - Origin of the case;
   - Background information;
   - Additional sources.

3. Information for the group discussion:
   - Background information which, if preferred, may be given to the discussion leader only;
   - Suggestions for the discussion:
     - Theme 1: Critical view of the hazards and risks.
     - Theme 2: Expanding the safety perspectives.

4. Wrap up:
   - Summary.
   - Suggestions for concluding the discussion.
   - Options for 'affective learning'.

Table 2. Example of case break down per item and time allocation.

What	Focus	Who	Duration
General introduction and goal/s	Necessity of safety when working with biotechnology	Discussion leader	5 mins
Case presentation and space for explanatory questions to be asked	Case description	Discussion leader or group member	10 mins
Group discussion: domain-specific safety	Hazards, risks and risk reduction measures		20 mins
Group discussion: general factors influencing safety	Extending safety to SbD	Either plenary or in small groups	20 mins
Conclusion	Lessons learned	Either plenary or in small groups	5 mins
Total duration			60 minutes

The case material can be used in whatever way you see fit: ranging from direct application of the provided suggestions to be used as triggers for discussion, to input from your own experiences to stimulate discussions. The information and suggestions can be seen as background information and inspiration and do not need to be copied literally.

2.4. Overview of the cases

In Table 3 an overview is given of the five cases and the particular biotechnological technique that is addressed.

The cases offer a wealth of information. To provide a focus we have preselected particular angles to be highlighted in the discussion. Each of the cases focuses on different aspects. Feel free, however, to tailor the questions to your liking.

Table 3. Overview of the cases.

Case number	Case topic	Description summary
1	Auxotrophy	A cyanobacterium is genetically modified in a way that enables it to grow on the nutrients melamine and phosphite. Both nutrients are not available in the natural environment. As a result, the cyanobacterium cannot survive outside its containment. This nutrient dependency can serve as biocontainment strategy for the genetically modified (GM) cyanobacterium.
2	Orthogonality in the genetic code	Orthogonality refers to biological systems whose basic structure is dissimilar to those occurring in nature. Here, the backbone of DNA is redesigned and composed of non-natural linkages. The DNA can still be replicated and used for gene expression in a living cell. This development raises questions about safety and future possibilities.
3	Converging techniques	A new technique has been developed making use of carbon nanotubes for DNA delivery to plants; more specifically to the plant chloroplasts. The ease of use of the nanotubes and their absence in the next generations of plants could be regarded as an advantage. However, carbon nanotubes are toxic and persistent in the environment.
4	Genetically modified bacteriophages as therapeutic	This case describes the selection of a three-phage cocktail for the therapeutic use of a human mycobacterial infection. One of the phages is genetically modified. Application to a patient is reported. In addition to the safety aspects, moral and ethical issues are raised.
5	Combining genetic parts	To study a virus that is only infectious to humans, a chimeric virus was constructed in order to facilitate animal studies in mice. The chimeric virus was a hybrid of Hepatitis B virus and Adenovirus. Broadening the host spectrum of a virus raises serious safety questions.

3. Background information on safety and Safe-by-Design

Why address safety and Safe-by-Design in biotechnology?

You may want to have a broader picture of the relevance of safety. If so, this information could be helpful:

- In order to address societal challenges in a sustainable way, future professionals, researchers, policy makers and entrepreneurs have to be aware of certain issues and/or areas to consider. In this context Responsible Research and Innovation (RRI) provides areas of interests when doing research[2] as does the Dutch 'Movement for New Economy Entrepreneurs[3](Maatschappelijk Verantwoord Ondernemen) (MVO).
- Safety is one of the areas to be considered[4], and refers to a safe and healthy living environment. Safe-by-Design is a safety approach that promotes critical reflection on safety issues in terms of hazards, risks and risk reduction measures early on in the innovation chain. Additionally, it challenges us to broaden the safety horizon with other perspectives to include societal, financial, socio-economic and ethical issues. In order to get this comprehensive view, relevant stakeholders and their views and roles are included in the SbD process. This is done from the earliest stage possible and in all stages from development to production, use, recycling and waste in a comprehensive way. This approach implies an interdisciplinary and cooperative way of working.
- In the field of biotechnology, developments in bioinformatics and genome editing (Clustered Regularly Interspaced Short Palindromic Repeats, CRISPR technology) have accelerated the possibilities for innovative applications of modern biotechnology. With synthetic biology providing biological building blocks, modern biotechnology is developing into an engineering discipline with a huge innovation potential. The array of applications is wide and includes both the traditional fields in agriculture, medicine, industry and newer fields such as energy and bioremediation. These (potential) applications 'attract' all kinds of new users, from engineers to hobbyists. They can relatively easily apply synthetic biology, often without the extensive knowledge and (safety) training of 'regular' users.
- All these (future) products and applications are intended to have benefits. At the same time, they may raise (new) serious questions about the safety of the activities in the production facility and beyond. These safety concerns challenge the scope and method of applying the existing policy and risk assessment frameworks. The comprehensive SbD approach could, therefore, enhance and promote safety in the design phase in this fast-moving promising field.

[2] https://ec.europa.eu/programmes/horizon2020/en/h2020-section/responsible-research-innovation

[3] https://www.mvonederland.nl/en/homepage/

[4] https://www.rivm.nl/en/about-rivm/knowledge-and-expertise/strategic-programme-rivm/2019-2022/safety-and-security

Examples of where a comprehensive approach is obvious are the introduction of gene drive and germline editing, both uses of CRISPR-Cas.

The cases presented are primarily focused on training students and researchers to critically reflect on the hazards, risks and risk reduction measures that can be applied in biotechnology and to expand their scope to include other issues that may relate to safety (e.g. societal, financial, socio-economic and ethical issues). They introduce a wider perspective on the systems, the various stakeholders and their (possible) role(s) in the process.

When discussing the cases it is important to have some knowledge about the current safety regulations in modern biotechnology. We have summarised the most important legal notions below.

The field of modern biotechnology has a long history of stimulating safe practice, which started with the initial landmark Asilomar recommendations issued in 1975. From that day up until now, policy and risk assessment frameworks have been developed based on the Asilomar recommendations. International organisations such as the OECD have also contributed to safe practice [5,6,7], establishing general principles and recommendations which are currently still in use across the globe And, in the EU, a strict regulatory framework[8] has been put into place. The main notions are:

a. When working in a laboratory, animal facility or green house, the setting is 'contained'. Even use of a large production plant is called contained use. For all these activities a notification or permission is required to comply with the regulatory framework[9]. The activity is permitted when the risks are assessed and managed to prevent any adverse effects impacting human health and the environment. For contained use four biosafety levels are in place (see Table 1).

Table 1 The safety levels, with examples of when applied.

Safety levels
1. Biosafety Level 1 (BSL-1, ML-I) for the genetic modification of e.g. the apathogenic bacterium Escherichia coli K12 while inserting a non-hazardous gene;
2. Biosafety Level 2 (BSL-2, ML-II), for the genetic modification of a pathogenic microorganism or virus that causes mild symptoms and/or for which a treatment is in place. E.g. foodborne pathogens or the adenovirus that causes a cold;
3. Biosafety Level 3 (BSL-3, ML-III), for the genetic modification of a pathogenic microorganism or virus that causes severe symptoms and/or for which a treatment is in place. E.g. the tuberculosis causing bacterium or the SARS-2 coronavirus;
4. Biosafety Level 4 (BSL-4, ML-IV), for the genetic modification of a pathogenic microorganism or virus that causes severe symptoms and for which no treatment is in place. E.g. a human pathogenic virus that easily spreads and for which no treatment is available. E.g. Ebola virus.

[5] OECD (1986), Recombinant DNA Safety Considerations, http://www.oecd.org/sti/emerging-tech/40986855.pdf

[6] OECD (1993). Safety Considerations for Biotechnology: Scale-up of Crop Plants, http://www.oecd.org/dataoecd/26/26/1958527.pdf

[7] OECD (1992), Safety Considerations for Biotechnology, http://www.oecd.org/dataoecd/8/3/2375496.pdf

[8] https://ec.europa.eu/food/plant/gmo/legislation_en

[9] https://www.ggo-vergunningverlening.nl/wetgeving

b. When applying a product of biotechnology outside the laboratory, a stepwise system must be followed. The regulatory framework requires that, before putting a product of biotechnology on the market, experiments must first have been done in a less contained environment. For genetically modified (GM) plants this means that after testing plants in a greenhouse (contained use), the plants must then be tested in a field trial (known as a deliberate release), and when all the data are collected regarding safety, the product can be placed on the market. In this last step, data regarding traceability and labelling are also required.
As well as the regulations regarding the safe use of biotechnology[10],[11], there are also regulations concerning the ethical issues in place. Examples of regulations that consider ethical aspects are: the law regarding the use of human embryos and the law regarding animal welfare; the use of certain micro-organisms is also regulated by quarantine measures and/or export controls.

Safety, hazards, risks and risk reduction measures

The cases address biotechnological safety concerns. Depending on the prior knowledge of your group members on this topic, you may want to elaborate on the biotechnology safety framework where hazards, risks and risk assessment are key to risk reduction measures. Below we describe these concepts and their relations, as used in the risk assessment framework for modern biotechnology.

- Safety applied to modern biotechnology refers to a state with no acceptable or negligibly acceptable levels of risks to humans and the environment.
- To gain insight into environmental and health risks one needs to assess the hazards: the potential adverse effects that could arise from the biotechnology. Subsequently the risks: the probability[12] of a hazard occurring, and the severity of its effect, can be assessed. If the risks are assessed, risk reduction measures can be determined and taken.
- When using modern biotechnology in the laboratory, it is necessary to take the following hazards into account:
  - the diseases to humans, animals or plants and the deleterious effects if the diseases cannot be treated;
  - the deleterious effects due to the establishment or dissemination in the environment of a Genetically Modified Organism (GMO);
  - the deleterious effects caused by the natural transfer of inserted genetic material into other organisms.
- Hazards may occur either in a setting of contained use or in a setting of semi or non-contained use. In case of the former, the hazards may occur during an incident, an unintentional release of the GMO from the lab or contained production facility in the surrounding environment. In a non- or a semi- contained setting the most common applications to date can be found when applying gene therapy or cultivating GM plants. However, new applications are likely to become more common. Some of these applications are algae farms, genetically modified (GM) fish farms, or the use of sensors using gene technology e.g. in remediation techniques for contaminated soil or to detect spoiled meat.
- Depending on the risks, the setting and control options, and the legal considerations, different risk reduction measures and/or strategies must be used. Furthermore the particular stages in the process of using modern biotechnology, from development to production, (re- or circular) use and waste, may bring different risks and call for different risk reduction measures/strategies.
- As the safe-by-design perspective values the involvement of stakeholders, students must be aware of the various stakeholders and their (possible) role(s) in the process - from identifying hazards, to risks, and developing risk reduction measures and their implementation. For instance, in one of the cases (Case 3), the need for expertise about nanotechnology means that nanotechnology experts should be included in the safety process right from the offing.

[10] https://www.ggo-vergunningverlening.nl/wetgeving/overige-wetgeving

[11] https://www.bvfplatform.nl/1732/aangrenzende-wetgeving.html

[12] The probability that a hazard occurs, and results in a risk, is subject to the GMO's characteristics, the environment, and the activity.

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