Dealing with risks of biotechnology: understanding the potential of Safe-by-Design

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About this report

The goal of this report is to provide an accessible summary of recent advances in biotechnology with regard to Safe-by-Design, a new way to deal with risks of biotechnology. The information presented is the result of literature review and ten expert interviews. The analysis in this report is rooted in philosophy and sociology of science. The report itself is written to reach a broad audience, as supported by the illustrations.

The author of the report, Dr. Zoë Robaey, is an ethicist of technology, focusing her research on ethical issues of biotechnology. Thanks to her interdisciplinary background in Biology, Science and Technology Studies, Public Policy and Ethics of Technology and work experiences in academia and the policy world, she strives to build bridges between disciplines and sectors to further the discussion around new developments. In addition to her work, Zoë regularly participates in public discussions on biotechnology and ethics and is a public speaker at events at De Balie, Pakhuis de Zwijger and Brainwash Festival. Zoë currently works at Delft University of Technology as a postdoctoral researcher in the Biotechnology and Society research group.

The graphic design work of this report is realized by Studio Michael van Kekem. Michael van Kekem works as an illustrative designer and printmaker. He has exhibited in galleries across Europe. His editorial illustrations are to be found in Gers Magazine. He has clients ranging from cultural institutions to governmental agencies and private businesses.

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All views in this report are of the author only.

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understanding the potential of Safe-by-Design

Executive summary
Safe-by-Design and Biotechnology

Safe-by-Design is an engineering concept for risk management. Recent rapid developments in biotechnology are possible with techniques that allow doing genetic engineering faster and with more precision. These developments cause new opportunities to arise such as eradicating diseases, breeding healthier livestock and cleaning soils. Questions on new risks of biotechnology follow these new opportunities. This report investigates and explains the opportunities and challenges offered by the concept of Safe-by-Design for managing these risks of biotechnology.

Amongst the techniques used in biotechnology, this report focuses on the ones used for genetic engineering. Some of these techniques have been around for longer and others are more recent. Concerns regarding risks are shared across these techniques. These risks can be formulated in four main categories:

1. Persistence, invasiveness and unintended effects on non-target organisms: This category of risk pertains to how a modified organism might change the balance in an ecosystem. This could mean that a modified organism could survive beyond the time it is supposed to, it could be stronger than expected and turn into an invasive species, or it could affect other organisms in an unexpected way. This can affect biodiversity and ecosystem functions.

2. Gene pool contamination: Another category of risk pertains to how modified organisms could change the existing genetic make-up of populations. This contamination could change biodiversity by bringing new individuals' genes into the gene pool.

3. Horizontal gene transfer: This category of risk contains risks mostly related to the use of bacteria but horizontal gene transfer is also suspected in more complex living organisms. It refers to the ability to pass on genetic material between individuals and perhaps even across species, which means that advantageous, or detrimental traits could be passed on with unknown consequences on the individual, or the ecosystem.

4. Pathogenicity and toxicity to other living organisms like plants, humans and animals: This

risk category contains risks surrounding pathogenicity or toxicity occurring in genetically modified organisms, i.e. making people, plants and animals sick.

In Safe-by-Design, design options for minimizing or preventing risks are defined early on and integrated in the design of a technology. In other words, Safe-by-Design brings assessing and managing risks closer through iterative design loops. In addition to being a risk management strategy, Safe-by-Design implies that we can design with safety as a design goal. Safety can be understood as the absence of unacceptable risks, but safety is also a public value, which involves the perception of safety by different stakeholders. Therefore, different experts and stakeholders may be involved in every iteration, by identifying risks and by defining what is necessary for safety.

Safe-by-Design, as a concept, has been implemented in engineering fields for a long time, without perhaps having been called as such. However, its application in biotechnology remains to be explored and elaborated upon. A simple example to understand safety in design is the seatbelt in your car; Implementing this design option enhances passenger safety.

In this report, Safe-by-Design is analysed at three levels: at the level of strategy, at the level of measures, and at the level of design options. Strategy is the way of dealing with a certain risk. Within a strategy, there are measures, i.e. specific approaches to execute a strategy. Design options are specific implementations of a specific measure. This analysis leads to explaining the concept of Safe-by-Design in biotechnology, examining its potential, and recommending further avenues of research attention.

Opportunities of Safe-by-Design

Strategies and measures for Safe-by-Design in biotechnology were identified through a literature review and expert interviews. These strategies are often labelled as biocontainment or inherent safety. So it is important to note that the terminology of Safe-by-Design is not yet overwhelmingly adopted by the biotechnology community.

As Table 1 below shows, a lot of research has already been done that can support the development of Safe-by-Design in biotechnology. The table lists the main strategies, their available measure(s) and the risks they address. A few important take home messages from this table are the following:

1. Most measures are applied and function at the level of the cell, or rather uni-cellular organisms.

2. Most measures address the risk of unwanted spreading of genetically modified organisms, rather than the subsequent risks that occur once the organisms have already spread in certain environments.

3. Most measures are developed for and applied in contained environments.

Table 1 Safe-by-Design strategies

Strategy	Measure	Risk scenario addressed
Choose the right organism	Not toxic	Toxicity of modified organism
	Not pathogenic	Pathogenicity of modified organism
	Environmental fit	Invasiveness of modified organism
Design physical barriers*	Physical containment	Spread of modified organism
Design a self-destruct mechanism*	Kill-switches	Spread of modified organism
Design a dependency*	Auxotrophy	Spread of modified organism
Design distance between the synthetic and the natural*	Orthogonality	Horizontal gene transfer
	Xenobiology	Horizontal gene transfer
	Recoding	Horizontal gene transfer
Sculpting evolution*	Daisy drives	Spread of modified organism and control for gene drive
Control with external stimuli*	Light	Spread of modified organism
	Temperature	Spread of modified organism
Design a warning mechanism*	Biosensors	Spread of modified organism

**These strategies have been researched and applied at the level of a uni-cellular organism.*

What are the next challenges of Safe-by-Design in biotechnology?

In order to make the most out of Safe-by-Design opportunities in biotechnology, five main challenges need to be addressed.

1 How safe is safe enough?

This challenge is both empirical, societal and political. How well do Safe-by-Design strategies work and how many measures are needed to ensure safety? How is safety defined and by which stakeholders? How do empirical and societal aspects of this challenge complement each other?

2 How do we deal with the complexity of living systems?

As most of the strategies are developed for and applied at the level of uni-cellular organisms, this is also where they have impact. In addition, most of these strategies are developed for contained environments. How will they fare in more complex organisms? Or in open environments? Are other strategies needed for more complex situations?

3 Which risks do we actually address with these strategies?

For the most part, the Safe-by-Design strategies aim at stopping the spread of genetically engineered organisms. This in itself allows the prevention of the risks in the broad risk categories mentioned earlier. While these strategies are effective, they are perhaps not specific enough to deal with more complex situations, where more specific measures are required. For example, cases where useful biotechnology innovations need to be released in the natural environment. These measures should facilitate using an innovation to benefit from it while minimizing the associated risks.

4 What are other strategies for realising safety?

This report examines technical strategies for safety. However, safety also involves risk assessments from different disciplines, and actions from different stakeholders. How can Safe-by-Design be supported in non-technical ways? Could other kinds of strategies look at the roles and responsibilities of experts and stakeholders to implement safety?

5 What about evolution and Safe-by-Design?

There is an inherent tension between designing and evolution. Designing for safety in living organisms is possible, but at what point will evolution make design obsolete? Perhaps, instead of taking controllability and predictability as design principles for Safe-by-Design, other principles could arise from taking evolutionary forces into account.

What's next for Safe-by-Design?

As next steps for Safe-by-Design, more empirical research is needed to understand the risks we might face when using biotechnology applications and to understand how well the design strategies work in practice. In addition, the understanding of Safe-by-Design as a design process that involves experts from different disciplines, stakeholders, and iterations to the design of a biotechnology application is fundamental and needs to be expanded. Additionally, the design process itself needs to be streamlined. Last but not least, given the vast potential of biotechnology, a steering force is needed to direct future research efforts.

Managementsamenvating

Safe-by-Design en biotechnologie

Safe-by-Design (inherent veilig ontwerpen) is een ontwerpprincipe voor risicomanagement. Recente ontwikkelingen zorgen ervoor dat biotechnologie steeds preciezer en sneller wordt. Hierdoor ontstaan nieuwe mogelijkheden voor bijvoorbeeld het uitroeien van ziektes, het gezonder maken van het vee en bodemsanering. Tegelijkertijd rijzen bij deze nieuwe mogelijkheden vragen over de risico's van biotechnologie. Dit rapport onderzoekt de mogelijkheden en uitdagingen rondom het concept Safe-by-Design voor het beheersen van de risico's van biotechnologie.
Van alle technieken die gebruikt worden binnen de biotechnologie richt dit verslag zich specifiek op de technieken die zich richten op genetische modificatie. Sommige van deze technieken bestaan al langer, anderen zijn recenter. De zorgen over de risico's gelden voor al deze technieken.
Deze risico's zijn grotendeels in te delen in vier hoofdcategorieën:

1. Persistentie, invasiviteit en onbedoelde effecten op andere organismen dan het doelorganisme: de eerste risicocategorie heeft betrekking op hoe een gemodificeerd organisme de balans in een ecosysteem zou kunnen verstoren. Bijvoorbeeld als een gemodificeerd organisme langer leeft dan het zou moeten leven, sterker is dan bedoeld en daardoor een invasieve soort wordt, of het andere organismen beïnvloedt op een onverwachte manier. Dit heeft effect op de biodiversiteit en op ecosysteemfuncties.

2. Genenpool vervuiling: een andere risicocategorie die betrekking heeft op hoe gemodificeerde organismen de bestaande genetische verzameling van een populatie kan doen veranderen. Deze vervuiling kan de biodiversiteit beïnvloeden of veranderen door het introduceren van nieuwe organismen in de genenpool.

3. Horizontale genoverdracht: deze risicocategorie heeft vooral betrekking op bacteriën, maar zou ook kunnen voorkomen bij complexere organismen. Dit betreft de mogelijkheid om genetisch materiaal over te dragen tussen individuele organismen en misschien zelfs tussen soorten. Daardoor kunnen voordelige, of wellicht nadelige eigenschappen overgedragen worden met onbekende

consequenties voor het individu of voor het ecosysteem.

4 Pathogeniteit en toxiciteit voor andere levende organismen zoals planten, mensen en dieren: deze risicocategorie omvat de risico's rondom pathogeniteit en toxiciteit veroorzaakt door genetische modificatie van bekende organismen. Dit wil zeggen dat ze mensen en dieren ziek kunnen maken.

Bij Safe-by-Design worden ontwerpmogelijkheden die risico's verkleinen of voorkomen vroeg in het ontwerpproces geïdentificeerd. In andere woorden zorgt Safe-by-Design ervoor dat het beheren en beoordelen van risico's wordt meegenomen in een iteratieve ontwerplus. Behalve als risicomanagement-strategie, kunnen we Safe-by-Design beschouwen als een manier om te ontwerpen met veiligheid als (meetbaar) ontwerpdoel. Veiligheid kan enerzijds worden gezien als de afwezigheid van onacceptabele risico's. Anderzijds is veiligheid ook een maatschappelijke waarde, waarbij de perceptie ervan door verschillende stakeholders van belang is. Daarom kunnen bij elke iteratie verschillende experts en stakeholders worden betrokken voor het identificeren van risico's en bij het bepalen wat noodzakelijk is om veiligheid te waarborgen.
Het concept van Safe-by-Design is al vaak toegepast in verschillende technische disciplines, misschien zonder expliciet zo genoemd te worden. Een gemakkelijk voorbeeld om te snappen wat Safe-by-Design betekent, is de autogordel; die heeft niet altijd bestaan en was niet altijd wettelijk verplicht. Door deze aanpassing van het ontwerp is de veiligheid van passagiers verhoogd. Echter, de toepassingen bij biotechnologie moeten nog grotendeels worden ontdekt en uitgebreid.
In dit rapport wordt Safety-by-Design onderzocht op drie niveaus: op strategieniveau, op maatregelniveau en op het niveau van ontwerpmogelijkheden. De strategie is de manier waarop er wordt omgegaan met een risico. Onder een strategie hangen maatregelen, oftewel de aanpak waarmee een strategie wordt uitgevoerd. Ontwerpmogelijkheden zijn ten slotte de specifieke toepassingen van een bepaalde maatregel. Op basis hiervan kan het concept van Safe-by-Design in de biotechnologie worden uitgelegd, de potentie ervan worden bestudeerd en wordt het mogelijk om nieuwe onderzoeksrichtingen te identificeren.

Mogelijkheden van Safe-by-Design

Om strategieën en maatregelen voor Safe-by-Design in de biotechnologie te identificeren is

literatuuronderzoek uitgevoerd en zijn experts geïnterviewd. De gevonden strategieën worden vaak bestempeld als fysieke inperking of inherente veiligheid. Het is dus belangrijk om op te merken dat de term Safe-by-Design nog niet breed is omarmd door de biotechnologie-gemeenschap.

Zoals te zien in Tabel 1 hieronder, is er al veel onderzoek gedaan dat de ontwikkeling van Safe-by-Design in de biotechnologie kan ondersteunen. De tabel bevat de belangrijkste strategieën, hun beschikbare maatregel(en) en het risico waar ze zich op richten. Enkele belangrijke boodschappen uit deze tabel zijn:

1. Het gros van de maatregelen werken op het niveau van de cel, of liever gezegd de eencellige.
2. Het gros van de maatregelen richt zich op het risico van de ongewenste verspreiding van gemodificeerde organismen. Niet op de risico's die optreden wanneer een organisme eenmaal verspreid is in bepaalde omgevingen.
3. De meeste maatregelen zijn ontwikkeld voor en gebruikt in ingeperkte omgevingen.

Tabel 1 Safe-by-Design strategieën

Strategie	Maatregel	Verwant risicoscenario
Kies het juiste organisme	Niet toxisch	Toxiciteit van het gemodificeerde organisme
	Niet pathogeen	Pathogeniteit van het gemodificeerde organisme
	Ongeschikt voor de omgeving	Invasiviteit van het gemodificeerde organisme
Ontwerp fysieke barrieres*	Fysieke inperking	Verspreiding van het gemodificeerde organisme
Een zelfvernietigingsmechanisme inbouwen*	'Kill-switches'	Verspreiding van het gemodificeerde organisme
Ontworpen afhankelijkheid*	Auxotrofie	Verspreiding van het gemodificeerde organisme
Ontwerp afstand tussen het synthetische en het natuurlijke*	Orthogonaliteit	Horizontale genoverdracht
	Xenobiologie	Horizontale genoverdracht
	Hercoderen	Horizontale genoverdracht
'Sculpting evolution'*	'Daisy drives'	Verspreiding van het gemodificeerde organisme en beperking van gene drive
Beheersing door externe stimuli*	Licht	Verspreiding van het gemodificeerde organisme
	Temperatuur	Verspreiding van het gemodificeerde organisme
Ontwerp een waarschuwingsmechanisme*	Biosensoren	Verspreiding van het gemodificeerde organisme

**Deze strategieën zijn onderzocht en toegepast op het schaalniveau van eencelligen.*

Wat zijn de volgende uitdagingen van Safe-by-Design in de biotechnologie?

Om Safe-by-Design mogelijkheden zo goed mogelijk te benutten in de biotechnologie, moeten vijf uitdagingen worden aangepakt.

1 Hoe veilig is veilig genoeg?

Deze uitdaging is empirisch, maatschappelijk en politiek tegelijk. Hoe goed werken Safe-by-Design strategieën en hoeveel strategieën moeten we toepassen voordat veiligheid voldoende gegarandeerd wordt? Hoe wordt veiligheid gedefinieerd en door welke stakeholders? Hoe vullen de empirische en maatschappelijke aspecten van deze uitdaging elkaar aan?

2 Hoe gaan we om met de complexiteit van levende systemen?

De huidige strategieën zijn vooral ontwikkeld en toegepast op (het niveau van) eencellige organismen. Daarnaast zijn de meeste strategieën ontwikkeld voor gebruik in ingeperkte omgevingen. Zullen ze ook werken voor complexere organismen? Of in een open omgeving, i.e. in het milieu? Hebben we andere strategieën nodig als de situatie complexer wordt?

3 Welke risico's pakken we aan met de huidige strategieën?

De huidige Safe-by-Design strategieën richten zich vooral op het blokkeren van de verspreiding van genetisch gemodificeerde organismen. Deze blokkade voorkomt de risico's in de eerder genoemde risicocategorieën al. Hoewel effectief, zijn dit geen specifieke strategieën. In complexere situaties kunnen specifiekere maatregelen nodig zijn, bijvoorbeeld in het geval dat biotechnologische innovaties in het milieu worden geïntroduceerd. Deze maatregelen moeten ervoor zorgen dat men baat kan hebben bij een innovatie door de aanverwante risico's zo klein mogelijk te maken.

4 Wat zijn andere strategieën voor het realiseren van veiligheid?

In dit rapport worden vooral technische strategieën voor veiligheid bestudeerd. Echter, veiligheid heeft ook te maken de waardering van risico's vanuit verschillende disciplines en hangt ook af van het

handelen van verschillende stakeholders. Hoe kan Safe-by-Design worden ondersteund met niet-technische benaderingen? Zijn er strategieën denkbaar waarbij tevens gekeken wordt naar de rollen en verantwoordelijkheden van andere experts en stakeholders bij het implementeren van veiligheid?

5 Hoe zit het met evolutie ten opzichte van Safe-by-Design?

Er is een inherente spanning tussen ontwerpen en evolutie. Het ontwerpen van veiligheid in levende organismen is mogelijk, maar wanneer zal evolutie het ontwerp nutteloos maken? Wellicht ontstaan er andere ontwerpprincipes voor Safe-by-Design wanneer evolutionaire krachten in acht worden genomen, in plaats van de ontwerpprincipes van voorspelbaarheid en controleerbaarheid.

Wat zijn mogelijke vervolgstappen voor Safe-by-Design?

Een eerste volgende stap voor Safe-by-Design is het doen van meer empirisch onderzoek om te begrijpen met welke risico's we te maken hebben bij het gebruik van biotechnologische toepassingen en om te begrijpen hoe goed de ontwerpstrategieën werken in de praktijk. Het begrip dat Safe-by-Design een ontwerpproces is waarin verschillende experts en stakeholders, alsmede het gebruik van iteraties, een belangrijke rol spelen, is hierbij fundamenteel. Dit moet nog verder worden uitgebouwd en het proces van ontwerpen moet hierop worden ingericht. Ten slotte, gezien de enorme potentie van biotechnologie en van Safe-by-Design, is een sturende kracht nodig die toekomstig onderzoek richting kan geven.

How to read this report

If you are a policy-maker, this report gives you an introduction to the concept of Safe-by-Design, offers an accessible overview of the latest development of Safe-by-Design in biotechnology, and triggers reflection on the opportunities and challenges of this concept.

If you are an expert in the field of biotechnology, this report gives you another perspective on your field and potential challenges. It might trigger new ideas, or reaffirm your motivation for the direction of your research.

If you are a student in biotechnology, this report gives you a good overview of recent advances and challenge the way you might think about safety in biotechnology as future engineer or scientist.
If you are a member of the public interested in biotechnology and safety, this report gives you some basic yet in-depth insights into the field, as well as tools to reflect on them.

In addition, Annex II provides some useful definitions to support the reader in understanding recent advances in biotechnology.

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1 Introduction

1.1 The building blocks of this report

Safe-by-Design is an engineering concept that involves early decisions in design choices leading to the increased overall safety of an innovation. This concept has origins in inherently safer design in chemical engineering, electrical engineering, civil engineering, etc. Recently, emerging fields such as nanotechnology have been using and implementing ideas of Safe-by-Design. In different fields, Safe-by-Design might have different implementations. This report focuses on Safe-by-Design as a risk management strategy in biotechnology (van de Poel and Robaey, 2017).

Thanks to digital methods, the speed at which we are able to read, model and write the genome has drastically increased (Rerimassie et al. 2016). This has accelerated the rate at which biotechnology has uncovered, reproduced and controlled processes governing the living world. While these new developments may have brought new risks (COGEM and Gezondheidsraad, 2016), the same developments may allow building safety measures into the genome. For instance, the concept of Safe-by-Design has been introduced in synthetic biology in recent years, especially in the practices of the international competition for the Genetically Engineered Machine (iGEM) community. Safe-by-Design measures such as substrate specific modified bacteria, kill-switches, and encapsulation are common practices. These measures are examples of using Safe-by-Design as a risk management strategy. Chapter 2 introduces important concepts and examples to understand the idea of Safe-by-Design.

What is the current state of Safe-by-Design for modern biotechnology? Currently, the division of Applied and Engineering Sciences of The Netherlands Organisation for Scientific Research is funding a 'Biosafety' program under the heading of 'inherent safety', which is another way of describing Safe-by-Design approaches. This suggests that new technical solutions for Safe-by-Design are approaching, these may increase the safety of biotechnological innovations. Chapter 3 provides an inventory of strategies in biotechnology that can be understood as Safe-by-Design.

Why is there a need to speak of safety of biotechnological innovations? Several recent reports highlight the need for discussion about biotechnological innovations with great promises and the need to

deal with their risks (COGEM and Gezondheidsraad, 2016; SCENIHR, 2015). In addition, public consultation work commissioned by the Ministry of Infrastructure and Water Management shows that with respect to biotechnology, safety is an important value for the public (InSites Consulting, 2017). The products of biotechnology are living innovations, and their release in the environment must be safe. Is Safe-by-Design a promising avenue for achieving this? Chapter 4 discusses the challenges of Safe-by-Design strategies and Chapter 5 suggests avenues for further research.

Before delving into the topic, Chapter 1 will provide a very brief introduction to biotechnology. It introduces the main ideas needed for the non-expert to read this report by introducing basic notions of biology and biotechnology. It is by no means an exhaustive explanation of biotechnology but it will give the essential knowledge to understand what biotechnology builds on, what for and how it is used, and the main ideas behind managing its risks and benefits. This report is based on literature review of scientific literature, reports, and is illustrated by some recent news articles. In addition, ten expert interviews were carried out to discuss the issue of Safe-by-Design in biotechnology, a list of experts interviewed can be found in Annex I. Last but not least, the method of this report is a conceptual analysis anchored in ethics of biotechnology, ethics of engineering and ethics of risk.

1.2 The complexity of life

Doing biotechnology implies making a technological application with living organisms at its base. So unlike other engineering fields, biotechnology deals with living organisms, which have the capacity to reproduce, spread, and evolve. Life is complex not only by virtue of being ever-changing, but also in its diversity of organisms, the interactions of living organisms with their environment, and the many biological and chemical processes cooperating within an organism.

The living world comprises of micro-organisms, fungi, plants, and animals. Here, complexity is often linked to the size of the living organism's genome, its complete set of genetic material. For instance, a bacterium has a smaller genome than a pig (see Annex II for useful definitions). Complexity also represents the level of biological organisation at which we study a living organism, and the scope interactions with other organisms and the environment we take into consideration. Figure 1 depicts

this increasing complexity. We can either look only at its genome, or look at the level of the cell, or at the level of a community of cells, which could also make up an organ, and eventually a complex organism. Furthermore, one can consider the complex organism alone, or a population in its ecosystem.

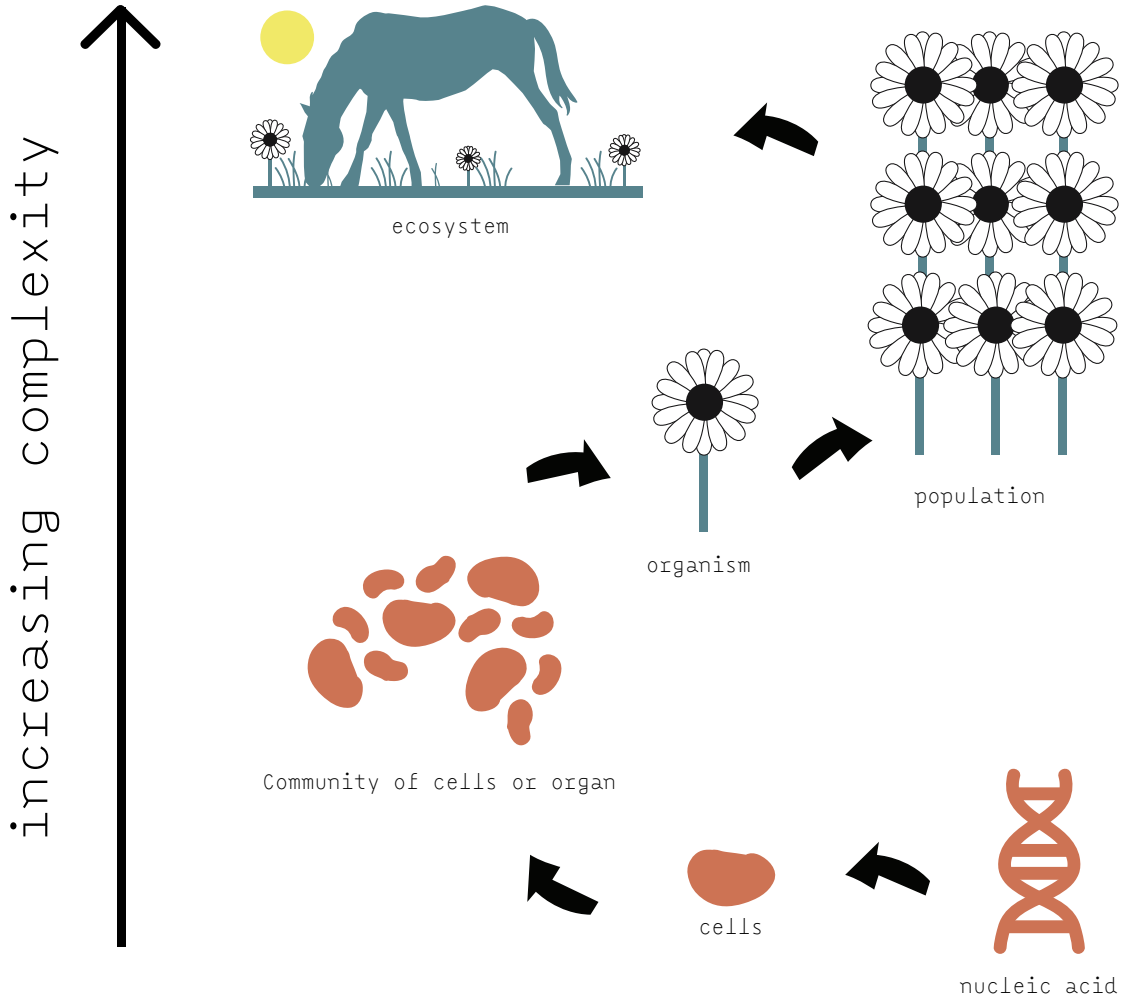

Figure 1: The complexity of life (Artist reproduction)

Since the discovery of deoxiribonucleic acid (DNA), many speak of it as the code of life. While life is diverse in its forms and in levels of organization, DNA is the great unifier. However, having DNA as a common denominator, does not make life less complex. The genome contains genetic information as DNA. DNA gets transcribed into mRNA which then gets translated into specific peptides. These peptides can be combined to form proteins, which ensure basic functions of life. These will vary according to the organism and its needs.

In other words, everything that allows an organism to function can be retraced to its DNA. In this report, if you read about the expression of a trait, characteristic, or function, you can remember that sequences of DNA were transcribed, translated, and assembled so that this information (trait, characteristic, or

function) could be expressed.

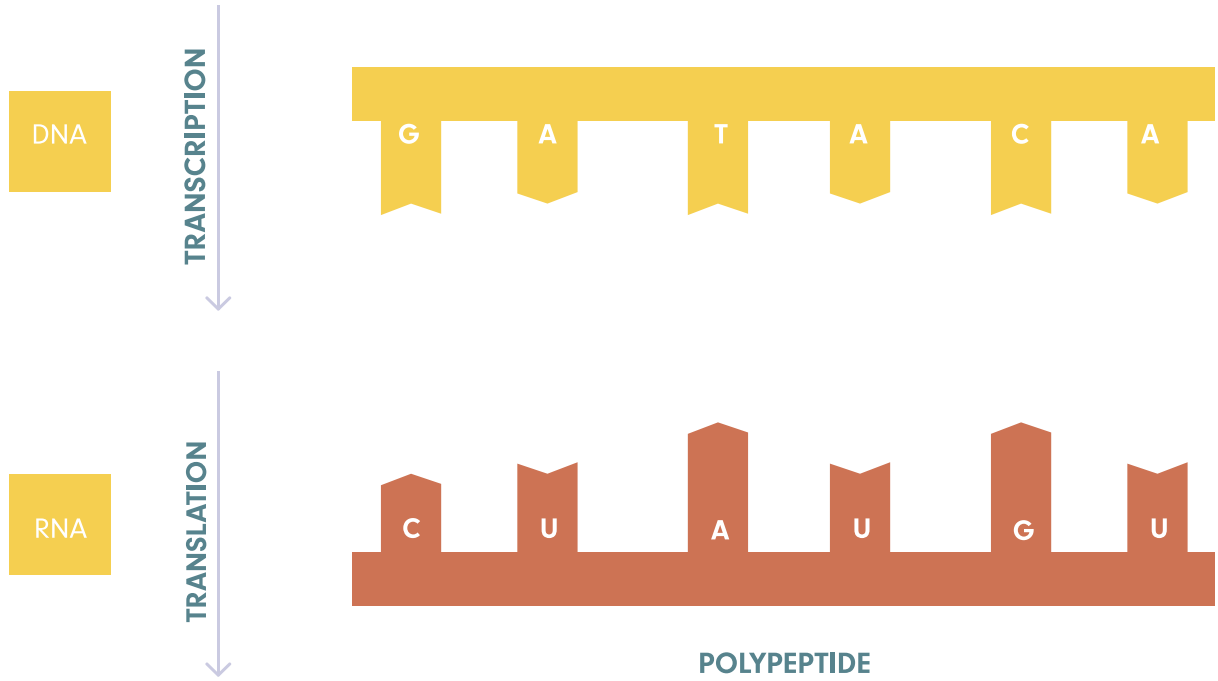

Figure 2: The code of life (Artist reproduction)

What is important for the reader to remember is the following:

Life starts with DNA and DNA contains information that allows for traits, characteristics, or functions of an organism.

However, while life starts with DNA, DNA is not all that is needed to understand life. Living organisms are complex due to interactions with the environment and other organisms.

Living organisms can reproduce, spread, evolve and die.

Understanding these processes and complex interactions is the realm of biology research, while using this knowledge in application is the realm of biotechnology.

1.3 The idea of biotechnology

While biology is the study of the living world, biotechnology is an engineering discipline that applies knowledge of biology to steer the living organisms in order to exploit their functions, or functional properties, for the benefit of humankind. Exploiting functions can mean using existing functions, and adding or removing functions. Properties or characteristics can be seen as synonyms for function here.

Here are some examples of biotechnology: plants with medicinal properties like the neem tree, new varieties of apples, or your favourite beer.

Exploiting functions of an organism can be applied in at least these three ways:

Extracting a component from a living organism, like with the neem tree which contains components with antifungal properties. In that case, biotechnology is closely linked to chemistry and biochemistry.

Managing the functions of a living organisms for processing, like fermentation for beer production. Fermentation is possible thanks to yeast, a micro-organism. There is not one type of yeast but many types of yeasts, that are called strains, many of which happen to be domesticated for processes like beer making (Callaway, 2016). This process of domestication happened through training and selection.

Designing organisms like with new varieties of apples. Here, choices are made in crossing different apples in order to get a sweeter taste or a smaller size. This is the basic idea behind farming: most plant varieties or animal species that we commonly consume today did not exist in the wild, they were bred for consumption because they had particular properties that were desirable. Here functions can be understood as taste, nutritional value, colour.

The above-mentioned examples can be considered traditional biotechnology. Today, we speak of ***modern*** biotechnology when we refer to biotechnology that makes use of rapid, precise, and targeted methods (Rerimassie et al, 2016). With the advance of techniques, the main activities of biotechnology can remain classified under those three main activities described above: extracting, managing and designing, and another category can be added, the one of transforming. Modern techniques can replace traditional means to perform aforementioned activities, with new implications. In other words, modern techniques can allow doing things we could never do before.

Extracting with modern biotechnology has been made easier thanks to targeted new techniques allowing identification and extraction of useful sequences. The faster we learn about useful sequences, the better we can specifically extract them for various applications. This can be seen in applications for rapid diagnostics, where instead of doing lengthy and costly procedures, targeted

information of DNA allows the identification of relevant traits much faster.

Managing with modern biotechnology means inserting information in the DNA that will manage the life of the organism by expressing a given trait, or restricting certain functions. An example of this are hybrid crops in agriculture where both plant parents are necessary to make a strong offspring. This allows to control hybrid seeds by lessening how well they might do in nature.

Designing organisms with modern biotechnology means that the extent of possibilities is expanded and therefore the extent of functions we might deem desirable. For instance, the Golden Rice, a rice designed to be enriched with vitamin A, could help fight preventable blindness due to vitamin A deficiency.

Transforming means that as a result of combining ideas of extracting, managing and designing organisms, one can engineer a cell as a factory which can produce, for instance, vanilla, biofuels, or insulin from different feedstock, thereby transforming raw materials into new materials.

Interestingly, with modern techniques to exploit functions, the boundaries between extracting, managing and designing organisms become blurred and allow for new activities such as transforming. For instance, being able to extract specific sequences of DNA can allow both managing, designing and transforming. Modern biotechnology, in this report, will refer to doing genetic engineering. There is much more to biotechnology than genetic engineering. However, given recent and rapid developments and corresponding safety issues, this report focuses on genetic engineering.

1.4 The techniques of genetic engineering

What are the main groups of techniques of modern biotechnology? In this section, five main approaches to doing genetic engineering are presented.

Trans-genesis involves techniques that allow inserting genes from a non-related organisms into another organism. The most famous case of trans-genesis are the Bt crops developed and commercialised by Monsanto. The Bt trait, or function, comes from the bacteria ***Bacillus Thurengesis***. Once inserted into a non-related organism, like cotton, or corn, it will be expressed and provide the plant with an inherent pesticide. This is an example of ***extracting*** sequences with a useful function, ***managing*** pests by engineering the management in the plant, and also ***designing*** a plant variety that

would have not existed otherwise.

Cis-genesis involves techniques that allow inserting genes from related organisms. This is an example where genetic engineering techniques allow doing the same thing than conventional ones but in a faster and more precise way. There are several examples of cis-genesis, one of the most famous recent one is a potato resistant to a devastating fungus called *Phytophthora infestans* (Schouten et al., 2006). Conventionally, you would breed different varieties when eventually by means of trial and error, you would be able to select a resistant potato that is also pleasant for the consumer. However, experience shows that this resistance does not remain long enough. Recent research has shown how thanks to cis-genesis a much more durable fungus-free potato can be bred, this constitutes designing an organism.

Synthetic biology understands DNA as building blocks that can be assembled to build a living machine. Biotechnologists who identify with this definition work mostly at the level of a micro-organism. Techniques in synthetic biology are not exclusive to synthetic biology, rather the approach of "programming" living machines is. Here, student teams participating in the International Competition for the Genetically Engineered Machine (iGEM) provides for a plethora of examples of the field (igem.org). The projects you find here are applied in all the ways mentioned in the previous section: ***extracting*** by doing targeted sequencing, ***managing*** by adding or removing sequences that enable or disable a micro-organism, ***designing*** organisms because these engineered machine would not occur by themselves, and ***transforming*** is perhaps the ultimate goal of many synthetic biology applications that create so-called cell factories. For instance, apart from being a fertilizer, phosphorus was long considered a pollutant to be removed from wastewater, but in the recent years, its removal and recovery has gained attention due to the decreasing availability of phosphorus from mines. Indeed, phosphorus is a resource that needs to be reused. Recent research outlines the role of synthetic biology in achieving this through genetically engineered machines that can capture phosphorus for it to be recovered (Beier and Schneider, 2018).

Gene editing involves removing or moving parts of the genome. Different techniques are used to this end like Zinc fingers, TALENs, and the recently very famous protein CRISPR Cas9 (Adli, 2018). Gene editing is ubiquitous, it can be used in the context of living machines in synthetic biology, in agriculture, in medicine. Most of all, gene editing with CRISPR Cas9 claims to be very precise, cheap, and easy to use. Recent news report speak of its use in editing out disease in human embryo (Davis,

2018) and its potential to radically change the way we dealt with biotechnology is enormous (COGEM and Gezondheidsraad, 2016). Like for synthetic biology, many of the goals of biotechnology can be achieved by this approach.

Gene drive involves inserting a trait that will almost certainly be passed on to all individuals of a population. Normal Mendelian genetics leave a great deal up to chance and gene drive simply changes this game. A review paper named 'Cheating Evolution', (Champer et al.,2016) outlines the different systems that do gene drive. The result of these systems is exactly what the title of the article suggests: we can use them to determine the desired genetic make-up of a population as gene drive techniques pass on the modified traits to all offsprings, and not randomly with a 50% chance as normally happens. Gene drive can be considered a population management approach although it could also be described as an approach to designing organisms. The most prominent example is the use of gene drive to eradicate malaria.

In this report, biotechnology refers, unless explicated otherwise, to these main approaches of doing genetic engineering. While some are not very new, like trans-genesis and cis-genesis, they are still used in research and development, and they also have a history of safe use. Other approaches like synthetic biology are much discussed in research but not found frequently on the market. Gene editing and gene drive are the next frontier of biotechnology.

While the above descriptions simplify these approaches to make them understandable, it would be a mistake to understand them as simple, or easy. Indeed, these methods are useful for making use of the functions of DNA that we understand. There is, however, much of the DNA that we still do not understand. We also do not know what the synergistic effects of DNA sequences are on other sequences, or what the influence of the environment might have on certain DNA sequences. So doing genetic engineering is not easy, but it is becoming easier, as these approaches and techniques increasingly allow for modifying and understanding the genome.

1.5 The risks of modern biotechnology

What are the risks of these approaches in modern biotechnology? As explained in the previous sections, genetic engineering allows a gain of function, something useful to humans and that is why it is used.

Another aspect of genetic engineering is that these changes are performed on living organisms that have the capacity to reproduce themselves under the right conditions that might mutate, or evolve. This aspect leads to some suspected risks of using genetic engineering, i.e. a likelihood of undesirable events occurring.

How can we talk about risks of biotechnology in general? It is difficult because each goal of biotechnology, each approach, each technique, each organism, each modification will call for a precise investigation of potential risks. However, from a review of recent reports and the expert interviews, four main types of risks can be identified.

Persistence, invasiveness, or unintended effect on non-target organisms. If we were to change an organism, would we inadvertently make it fitter? If we were to introduce a modified organism to an existing environment, would it strive to the detriment of other organisms? A modified organism could persist or become invasive. Invasive species might affect biodiversity. If we gave an organism a gain of function to defend itself, might it affect other organisms that were part of its ecosystem? This is the emblematic case of the monarch butterfly which was said to have become endangered following the introduction of genetically engineered crops in the US and Canada. Research has later shown a negligible impact, however, this claim led to much research attention (Sears et al., 2001). This case underlines that there is a risk that a modified organism could affect biodiversity and ecosystem services.

Gene pool contamination. Gene pool contamination refers to the idea that populations have a certain gene pool with a unique diversity. Adding modified organisms to a population can change the gene pool, and affect biodiversity. It can also affect agro-biodiversity: for instance, traditionally cultivated crops with a certain cultural importance to a people might become 'contaminated' with modified traits from related modified organisms (Garcia, 2017). The impact can also be economic, organic farmers might lose their certification if their fields become 'contaminated' with GMO crops (Robaey, 2016).

Horizontal gene transfer. Bacteria are most famous for exchanging genes; thanks to their size and lifespan, they can do this quite efficiently. One of the most famous examples of risk from horizontal gene transfer is anti-microbial resistance (Barlow, 2009). Indeed, microbes will share genes that allow them to resist antibiotics, making it increasingly difficult to fight microbial infections. Anti-microbial resistance raises fears of eventually creating a 'superbug', immune to our available

remedies. Horizontal gene transfer is quite rare amongst members of the same species in more complex organisms.

Pathogenicity and toxicity to other living organisms. In 1974, the Asilomar conference put a moratorium on working with techniques of genetic engineering involving pathogenic organisms until regulation could deal with it. While risk assessment for biocontainment of pathogens involves complex considerations (Patterson et al. 2014), working with genetic engineering techniques raises worries of potential gain of function that might result in pathogenicity or toxicity, i.e. making other humans, animals and plants sick. For instance, novel foods[1] have raised potential concerns with regard to allergic reactions, a form of toxicity (Meredith, 2005).

These main types of risks do not actually fit the definition of risks mentioned above, i.e. they do not describe a likelihood for undesirable events. Rather, these types of risks might be better defined as potential hazards, or the suspicion of undesirable events, without any probability associated with it. Once a specific modified organism is being studied for a given context, one can perhaps refine these potential hazards in terms of risks and devise ways for how these can be managed, on a case by case basis.

1.6 Benefitting while minimizing risks of biotechnology

Managing risks is indeed an important way for societies to benefit from many things, including advances in biotechnology. For instance, having traffic signs, or wearing a seatbelt in a vehicle are examples of how we manage the risks of driving a car and enjoying the benefits of mobility. Risk management strives to realize risk minimization. What does risk management look like with biotechnology?

Two situations are relevant when managing risks of biotechnology: is the modified organism in a contained environment, or is it released in a non-contained environment?

At the moment, the main type of released modified organism is the genetically modified seed in agriculture. Genetically modified seeds represent a growing market in agriculture (ISAAA, 2017) but their use remains controversial. In order to manage their use, many countries require a lengthy risk assessment process. Once approved, things like buffer zones and refuges for pest control allow managing identified risks of using them. There are differences in how countries choose to deal with

1 | In the context of biotechnology, a novel food can be a never eaten before food, like a completely new GMO food, or food that are the result of genetic engineering, like a new flavour additive developed through synthetic biology

those risks. Differences in approach between the European Union and the United states have been maintained since the early 2000s (Löfstedt and Vogel, 2001).

Besides release, the other situation is containment, which means using the modified organism in a way that it will never mix with the outside world, thereby avoiding any possible worries of the above mentioned types of risks. This approach is very safe but also greatly limits the realm of possibilities in applications. For instance, it would not be possible to use a modified organism that would remove and recover phosphate from wastewater when following this approach.

New techniques allow for new possibilities in biotechnology. What is the future of risk management for genetically engineered organisms? In this report, the concept of Safe-by-Design is explored in relation to risk management of biotechnology.

2 Concepts of Safe-by-Design

Chapter 1 underlines that new approaches and techniques in biotechnology call for new ways of managing risks. This report explores Safe-by-Design as one possible way of doing so. Chapter 2 introduces the concept of designing for values, and then more specifically designing for safety. To understand how this could be fleshed out, experiences from three other fields - nanotechnology, pharmacology and chemical process engineering - are presented. Together, these experiences allow to gather some general lessons about Safe-by-Design before taking an in-depth look at its possible application in biotechnology.

2.1 Designing for Values

To design means to make choices about the function of a system, or a human-made object. In recent scholarship in the ethics of technology, coming from the field of human-computer interactions, Value Sensitive Design is an approach and method to integrate human values in design (Friedman et al. 2006). Recently, van de Poel (2013) has formulated an approach to link design requirements to norms and values. Take for instance the value of sustainability: Leadership in Energy and Environmental Design (LEED, 2018) standards promote building norms that help reduce and conserve energy production, and contribute to clean air. Another example can be the value of resilience: recent hurricanes have devastated coastal areas, few houses were left standing because they were designed to withstand extreme weather events (Mazzei, 2018).

Before delving into what it can mean for biotechnology, the following example will help understanding the different aspects of Value Sensitive Design.

To explain this, let us leave morals aside for a moment and imagine it is raining outside and you want to stay dry because you see it as desirable for your well-being. There are two strategies one can think of: avoid the rain, or repel the rain. For the strategy of avoiding rain, you could take the following measures: you could stay inside and wait until the rain has passed, or you could try to walk a route covered from the rain. For the strategy of repelling the rain, you could take the following measures: use an umbrella, or wear a rain coat. You could also combine strategies and measures by wearing a raincoat, carrying an umbrella and looking to walk in places that are covered. To further this, one could think of the different

options in terms of raincoats, umbrellas, routes, and weather apps to determine when the rain stops. These all have different means of achieving the same goal: staying dry.

Having a goal can be thus achieved or realized through different paths. Breaking down how to achieve this goal in strategies, measures and design options helps narrowing down which design options are appropriate in a given situation, or given certain preferences.

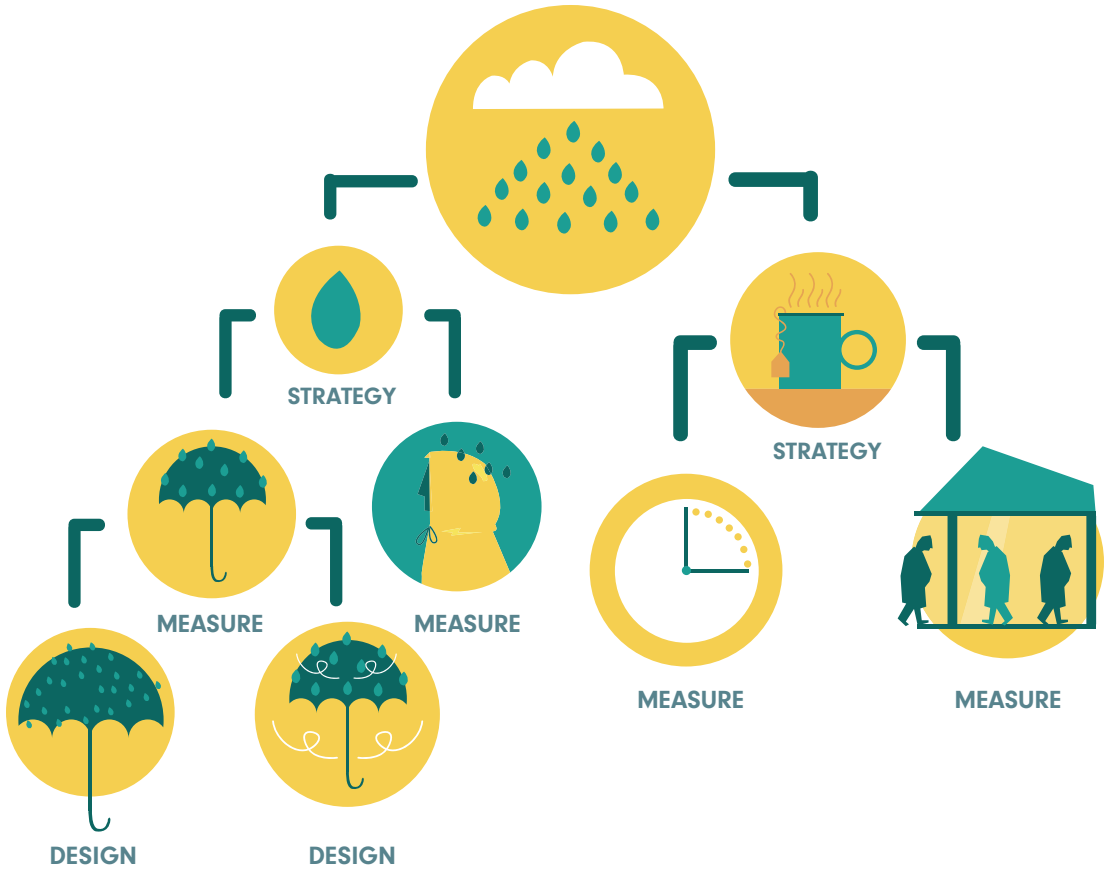

Figure 3: The relation between goal, strategies, measures, and design options

Let us now return to biotechnology and leave the rain behind. The goal we want to achieve with Safe-by-Design is safety, or avoiding or minimizing the risks presented in section 1.5. What does designing for safety mean in biotechnology?

2.2 Designing for Safety

Designing for safety means to make design choices that will lead to safety, where safety is understood as the absence of risks, or the minimization of risks (Robaey et al., 2017). This can be applied to the development of a technology by using certain safety design requirements. This is sometimes called Safe-by-Design and can be understood as a risk management strategy (van de Poel and Robaey, 2017; Schwarz-Plasch et al., 2017).

The concept of Safe-by-Design also relates to the idea of addressing potential risks early on. In the field of responsible research and innovation, this is tied to the ideas of anticipation and responsiveness (Stilgoe et al., 2013) and in the field of engineering ethics, methods from the field of value sensitive design can be used to realise Safe-by-Design through different steps of investigation (conceptual, empirical and technical) and an iterative design process (Friedman et al., 2006). Safe-by-Design can be understood as risk management strategy that includes an early risk assessment during design and design choices integrating a response to this assessment. Thankfully, designing for safety is not a new concept for managing and minimizing risks in engineering disciplines. In Chapter 1, the examples of signs on the road and seatbelts were already mentioned. The signs on the road are designed in the system of road traffic, and the seatbelt is designed in the car. Another example would be to avoid highly flammable materials when building a house to minimize risks of fire hazard. Those examples show risks like road accidents or fire, and how measures are built-in at the design phase to minimize the likelihood of these risks.

Safe-by-Design requires to identify potential risks during the design phase, identify a strategy to deal with the risk, develop measures, and formulate design options for safety.

When speaking of risk and safety, there are underlying notions of responsibility: who is responsible for making these choices and for risk assessment? In other words, who is responsible for safety, and in what sense? The underlying responsibilities are: a responsibility to prevent harm, i.e. a forward-looking moral responsibility (Robaey, 2015), and a way to hold people accountable in case things go wrong, i.e. a backward-looking moral responsibility. To stay with our car and building comparison, forward looking moral responsibility would be to install sensors on the car to stop it in time in case

of collision, or to use safe materials for building. The issue of backward looking moral responsibility gets trickier with new technologies, and with complex projects with many people involved. One can recall the discussions around who was responsible for the autonomous car killing a pedestrian in the US (Bogost, 2018) or who was responsible for the Grenfell fires in the UK (Booth, 2018). Given what we now understand about designing for safety, what are essential elements of Safe-by-Design? In the next section, these essential elements are teased out and illustrated with examples from other fields.

2.3 Doing Safe-by-Design

As mentioned before, designing for safety is not a new concept. After reviewing how Safe-by-Design or similar concepts are implemented in other fields, this section presents three interesting ingredients to what an implementation of Safe-by-Design should entail: formulating safe-by-design strategies, processes facilitating this formulation, and how these processes distribute responsibilities amongst stakeholders. These three aspects of Safe-by-Design as a risk management approach are important because we need to know how we can deal with a given risk (section 2.3.1), how we can come to ideas that will help dealing with that risk (section 2.3.2), and how we can distribute responsibility in a way that makes sense to stakeholders (section 2.3.3).

Earlier in this chapter, some example were given from the construction sector, that sector is however very different from the biotechnology sector. Which other fields have similar complexities such as the small physical scale of the technology, the difficulty to trace or see it, and its potential spread to the environment and other organisms? Examples are drawn from safety approaches in nanotechnology, chemical process engineering, and pharmacology as these fields share some of these complexities.

2.3.1 Formulating Safe-by-Design strategies

What kind of design strategies are formulated in other fields? Both in nanotechnology and in chemical process engineering design strategies are formulated in order to achieve safer products through production processes. These strategies allow for the formulation of more specific measures and design

options for safety. For instance, in nanotechnology, a strategy for Safe-by-Design is "Design out hazard (direct and indirect effects of nanomaterials)" (Kraegeloh et al., 2018). Designing out hazard means making choices inherent to the design of the nanomaterial, that make it safer. In chemical process engineering, various strategies can be identified for achieving an *Inherently Safer Design*, for instance to minimize the amount of chemical, or to substitute dangerous chemicals with safer alternative chemicals. All of these strategies lead to different choices early in the design process to enhance safety.

These examples suggest that an approach for Safe-by-Design in biotechnology could follow a similar reasoning: identifying strategies to deal with safety, and from there formulating measures and making design choices. Inherently safe design choices can already be made right at the start of a design process, so that it is not necessary to intervene later, For example by choosing a certain micro-organism to work with, similarly to choosing safer substances in chemical process engineering.

2.3.2 How can we formulate these strategies?

How do other disciplines come to formulate strategies for Safe-by-Design? Upon looking at processes in nanotechnology and pharmacology, two elements of their processes stand out as particularly helpful in formulating strategies: iteration and experimentation.

When it comes to iteration, one example comes from the use of the Stage Gate Model for the development of nanomaterials (NanoReg 2, 2018). At each gate, there is a decision moment guided by sets of criteria, if these are not fulfilled, the nanomaterial must return to the design table to fulfil them. These criteria include safety considerations. One possible challenge for biotechnology would be finding precise criteria at each gate. This is especially challenging because of the diversity of applications and situations.

Another example of iteration in the process can be seen in the process of *Quality-by-Design* in pharmacology, where critical characteristics of the drug and process parameters are defined, as a result of *in silico* (i.e. via computer modelling) and *in vitro* (i.e. laboratory experiments) testing (Lionberger et al., 2008; Yu et al, 2014; Pramod et al., 2016). While this is not Safe-by-Design, safety is an integral part of quality in this approach. Here, iterations are linked to various types

of experimentation. Experiments allow understanding a product, and formulating hypotheses about it in order to learn. Experiments allow to "fail early, and fail often" (Hjorth et al., 2017).

Iteration and experimentation facilitate learning about risks early and making decisions in design to address them at an early stage. Iteration and experimentation are therefore ingredients to formulating Safe-by-Design strategies that could be used in a Safe-by-Design approach in biotechnology.

2.3.3 Who should be responsible for formulating Safe-by-Design strategies?

Strategies for safe by design can lead to more precise design options. With the help of iterations and experimentations, these can be further defined. So who is responsible for doing Safe-by-Design? In the literature, Robaey and colleagues (2017) have suggested that Safe-by-Design in synthetic biology could place an excessive proportion of the responsibility for safety onto the designers due to these early decisions and assessment. This research has also suggested that doing so might be inefficient and unfair. How are responsibilities shared in other fields?

Given that there are still uncertainties regarding the unintended effects of nanomaterials, it might not always be possible to design for safety. Here, the need for learning and sharing information has led to the idea of trusted environments, where people working with nanomaterials can share worries anonymously in order to create a collective learning process (Kraegeloh et al., 2018). Another concept developed in the context of emerging technologies was the one of a societal incubator, creating a space for a collective learning process about emerging technologies and making choices about the development of a technology (van Lente, 2015; Rerimassie et al., 2018). Collective learning and sharing of information imply that the designer is not alone in her responsibility to define design options for safety. Indeed, others can and probably should contribute. Moreover, the process will require experts from other disciplines, experts at different stages of the innovation, and even lay experts to participate in formulating design options for safety. The key here is the need for interdisciplinarity. Similarly, in *Inherently Safer Design* for chemical process engineering, non-chemical engineering experts are involved.

Another way to distribute responsibility is also seen in *Inherently Safer Design* is but one of the many layers of risk management, such as control, supervision, safety culture, mitigation, and response. These layers can be seen as a way not to place the burden of safety only on those who practice *Inherently Safer Design*, but also after design like in use. Responsibility for formulating design options is not only attributed to the designer and can be shared in a collective made of different types of experts and at different moments in the development and use of the innovation.

Safe-by-Design offers a rich approach as a risk management strategy. What it can mean for biotechnology still needs to be shaped by the communities developing and assessing biotechnologies. Next, Chapter 3 goes in depth on available Safe-by-Design strategies identified in biotechnology as a first step towards understanding the potential of Safe-by-Design in biotechnology.

3 An inventory of Safe-by-Design strategies

In Chapter 2 underlines the elements that support a Safe-by-Design approach. The first aspect of doing Safe-by-Design is the formulation of strategies. What strategies for Safe-by-Design exist in biotechnology, and what are their measures? Before describing the existing strategies and corresponding measures, the following notions should be kept in mind:

1. The classification of strategies is done by the author of this report, as a way to synthesize the vast amount of information gathered and make it accessible to a general audience. In the field of biotechnology, these strategies are not classified as such.
2. The measures presented are general categories, meaning that within these measures, many options are available to implement them.
3. In Chapter 1, we began by looking at the complexity of life. While everything starts with DNA, life at every level exhibits a different level of complexity. This chapter also pays attention at which level of complexity the strategies and measures apply.
4. These strategies and measures are presented as stand-alone. However, just like with the example of 'staying dry' from Chapter 2, these may be combined or even mixed.

Strategy 1: Choose the right organism

Choosing the right organism is the first strategy identified. As we have seen, biotechnology is an engineering discipline that makes use of the living world. A number of questions arise while investigating this strategy, and each of these questions suggest measures. Does the organism have the potential to be toxic? Is the organism pathogenic? What are the ideal environmental conditions of the organism and how can we take these into consideration for enhancing safety? Answering such questions allows the exclusion of organisms that would have characteristics that can put human and environmental safety at risk, and allows for the selection of safe(r) organisms.

Illustration 1: Choose the right organism

Measure 1: Non-toxic organisms

Firstly and most important one might choose an organism that **does not produce toxins**. Toxins are poisonous substances produced by living organisms. In sufficient quantities, they can be harmful to other living organisms. Microbiologist Klaas Hellingwerf gives the example of cyanobacteria: some strains of cyanobacteria produce a neuro-toxin and other do not. If one wants to use cyanobacteria for a process involving susceptible organisms, then one should choose a strain that does not produce neuro-toxins. This inherently creates more safety.

Another example is mentioned by risk assessor Esther Kok in relation to allergenicity, which is a form of toxicity. It is important to assess the compounds intentionally produced by modified organisms, just like one would assess any new compound, for similarities with allergens, or other forms of toxicity. Avoiding toxicity therefore implies investigating the producing organism as well as the new compounds produced.

Measure 2: Non-pathogenic organisms

Secondly, one can choose organisms that are **non-pathogenic**. A pathogenic organism is one that is capable of making other organisms sick. Here, it is not the compound produced by the organism like with the toxin but rather the organism itself. One can recall outbreaks of E. Coli and recalled food. E. coli is a micro-organism that has about 45 strains. The ones used as a model organism for biotechnological

applications are non-pathogenic. The ones that cause outbreaks and food recall are pathogenic, and may also produce toxins. Risk assessor Cécile van der Vlugt mentions that scientists need certain clearances to work with different pathogenic organisms, and depending on these laboratories have different safety requirements. It is always desirable to work at the lowest possible biosafety levels. One can choose strains of an organism that are never pathogenic.

Measure 3: Ideal environmental requirements

Thirdly, thinking of the **environmental requirements** of a micro-organism can help choosing the right one. There are two aspects to this point: the environment the organism *needs* to thrive, and the environment in which the organism *should* thrive. By choosing an organism that needs environmental conditions only present in the place the organism is supposed to thrive, spreading of the organism is prevented.

As an example of the environment an organism *needs* to thrive, most model organisms we know thrive at mammalian body temperature. The most commonly used micro-organisms and best studied in biotechnology are probably E. coli and yeast. E. coli might be best known to the public in relation to food contamination outbreaks, but many strains live in human gut and are not dangerous, other strains are specifically cultivated for laboratory work and are also not dangerous. As we saw earlier in this report, yeast has been cultivated by humans for millennia for multiple fermentation processes, such as making bread or beer. It is not surprising that the best known micro-organisms are also the ones most commonly used as model-organisms (Cooper, 2000).

If we want to prevent the spread of a modified micro-organism to other organisms, such as humans, then choosing a micro-organism that does not thrive at body temperature, but that rather thrives at e.g. 15^{o}C or 50^{o}C, would greatly reduce chances of survival at mammalian body temperature. While there is nothing dangerous about the model organisms used for research, or industrial applications, as they are non-toxic and non-pathogenic (recall the first two points), one could think of expanding the realm of choices to other micro-organisms to apply this measure. Research suggests that there exist over a million species of bacteria in the world (Dykhuizen, 2005). As we expand our knowledge of the

bacterial world, we might become better equipped in the future at choosing organisms that help us fulfill safety goals by containing the modified organisms to specific environments.

When it comes to considering the environmental requirements where an organism ***should*** thrive, one should be aware of the ecosystem balance. In order to understand that this means, let us first recall the increasing complexity in which we can study life (Chapter 1), this measure requires thinking about the organism at the most complex level. The organism is part of a population and this population lives in an ecosystem. If we modify an organism and release it in an ecosystem, several questions arise (recall section 1.5 on risks). Would the modification change how the organism interacts with its environment? This can mean various things. The modified organism could become more or less dominant in relation to other organisms. The modified organism could behave differently with unexpected things in the environment, like maybe a plant, or a mineral. Adding or removing function to a modified organism may change more than what was targeted, and have unintended consequences on the environment.

Erik Joner mentions the importance of an organism's ecotype, a subgroup within a species' population that has become specific to a particular environment. This relates to thinking about the environment where an organism ***should*** thrive. For instance, modifying an organism could mean that it is similar to other organisms of the same species but it is no longer the same ecotype because of potentially unknown effects of the modification.

This is an issue similar to the one of invasive species. Historically, the seemingly harmless 24 rabbits brought to Australia in 1859 created one of the most famous cases of invasive species, calling for continued population control measures since rabbits have been adapting to them (Effler 2015).

For Safe-by-Design, the implication of thinking of the environment where an organism should thrive means that when choosing an organism, its intended environment should be well documented to anticipate and avoid undesired changes in interactions. This is an additional aspect of environmental requirements that can lead to different considerations in choosing the right organism.

Strategy 2: Design physical barriers

Designing physical barriers is possibly the second most obvious rationale of Safe-by-Design; it currently applies mostly to industrial settings. Where is the production site located? What are the walls of the factory made of? Where are the reactors in the plant? What are the reactors made of? For applications

in biotechnology, imagine creating a site and a reactor for micro-organism which would be the Alcatraz of bio-processing plants. Creating effective physical barriers can greatly minimize potential risks. This section presents two possible measures in this strategy: physical containment and encapsulation at the scale of the micro-organism.

Illustration 2: Design physical barriers

Measure 1: Physical containment

From a safety perspective, physical containment is an important design choice. However, heavy regulation of this form of containment does not always offer much choice. From an industrial biotechnology perspective, the concern is not so much that the micro-organism would escape, but rather that micro-organisms from the outside, or environmental factors would challenge the quality of production. Microbiologist Klaas Hellingwerf explains it as keeping the product safe, or stable, from the outside world. Physical containment in such industrial settings therefore seem to create both better quality products and enhanced safety. There might be room for innovation in reactors and industrial plants, that use processes harvesting natural light, such as Photanol BV[2], or BioSolar Cells[3], as these look to be built differently.

Measure 2: Encapsulation

How does physical containment play out at other levels than at industrial plant level? Much research has been done with regard to nano-encapsulation of probiotics. Probiotics are beneficial bacteria that can be added to foods like yogurt, in order to promote gut health. Researchers in this field seek to improve the delivery of probiotics to humans through nano-encapsulation, in this way probiotics do not

2 | https://www.photanol.com/ 3 | http://www.biosolarcells.nl/en/onderzoek/algen/

get lost along the way due to spoilage or destruction (Vidhyalakshmi et al., 2009). While the goal of this measure is not safety but rather stability of the product, it can serve a double purpose, just as seen in the previous measure. Encapsulation could prevent a modified organism to interact with other living organisms in the wrong place and at the wrong moment. The strategy to create physical barriers can lead to different measures and design options for safety, and while some of them might be well known, they might open the door for innovative solutions when considering different applications.

Strategy 3: Design a self-destruct mechanism

Self-destruct is often represented in movies as a red button to push in case of emergency. In movies, someone has the option to push the button when things go awry. Self-destruct is also a strategy in biotechnology. However, the red button is located in the genetic make-up of a micro-organism, which contains information that can induce cell death in certain circumstances. In order to push the red button, or to activate the expression of that information, external conditions have to change, or things have to go awry. The expression of that information leads to cell death, and thus eliminates the risk of spreading of the organism. This strategy is best described by one main measure: kill-switches

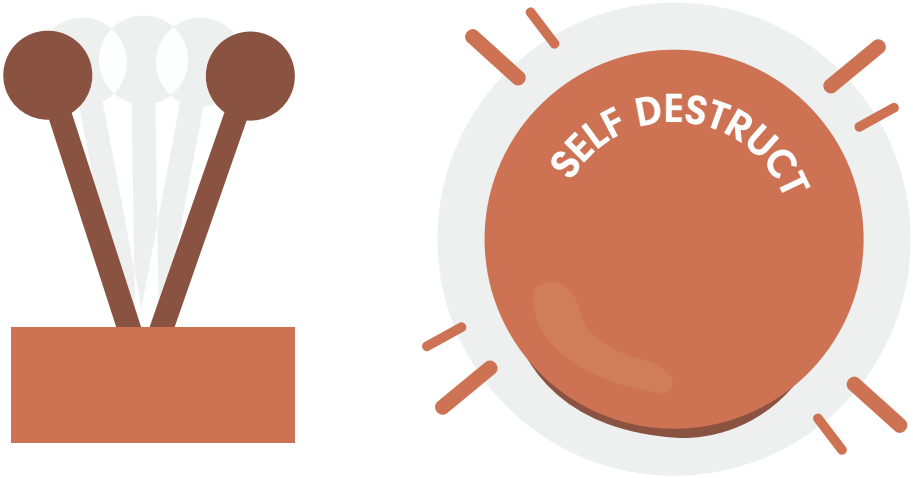

Illustration 3: Design a self-destruct mechanism

Measure: Kill-switches

Kill-switches are probably one of the most well-known strategies in synthetic biology. Their name refers to a mechanism that prevents car theft. Such analogy is not rare in synthetic biology as the field reproduces car analogies such as the chassis (Andrianantoandro et al., 2006). One of the leading scientists in synthetic biology, Drew Endy, writes, "for engineers, biology is a technology" (p.449, 2005). Synthetic biology is greatly inspired by electrical and mechanical engineering.

In synthetic biology, there is not one kill-switch but rather a great variety of kill-switches that can be activated through different mechanisms. Once the kill-switch is activated, it works with a toxin-antitoxin system (Harms et al., 2018). A toxin is a poisonous substance that can be produced by an organism, its antitoxin is its antidote. The expression of this toxin is prohibited by the constant presence of its antidote. The antidote in biotechnology can be a repressor, the presence of protein acting as an antitoxin, or as a piece of RNA that binds the toxin. Removing the antidote (or anti-toxin), leads to its expression of the poison (or toxin), which leads to cell death (Wright et al., 2013).

Today, kill-switches can almost be considered common practice in synthetic biology. For instance, in 2013 kill-switches were already regularly incorporated in the design of students' projects (Guan et al., 2013). Today, on the iGEM Registry of Standard Biological Parts, you can find 23 types of kill switches characterized by the community (iGEM, 2018)). In the recent scientific literature, kill-switches of names such as Deadman and Passcode (Chan et al., 2015) or Essentializer and Cryodeath (Stirling et al., 2017) are found, all of them using different mechanisms.

How efficient are kill-switches as a Safe-by-Design measure for biocontainment? The first pitfall of this measure is linked to fitness for survival of an organism. Engineered bacteria are living organisms, evolutionary forces will tend to not favour passing on the kill switch to future generations, since it does not favour the bacteria's fitness for survival. Recent kill switches like Essentializer and Cryodeath were able to remain present in the genome for 140 generations during *in vitro* experiments (Stirling et al., 2017). When compared to E. Coli growing at optimum rate this number of generations would amount to about two days of kill-switch containment.

The second pitfall regards lag phases and escape frequencies. A lag phase refers to the time it takes for a kill switch to be activated in a population of bacteria. Escape frequencies mean that out of a given population, there is a chance that the kill switch does not work in some individuals. For instance,

Cryodeath has an escape frequency of 1 in 10^5 after 10 days (Stirling et al., 2017), which means after a single bacteria escapes, it could reproduce itself exponentially.

In addition, research in recent years suggests that the self-destruct strategy can be applied beyond the cell itself, but also to cell communities targeting communication between cells (Agapakis et al., 2012; Shong et al., 2012). Bacteria can regulate gene expression linked to population fluctuation. This is called quorum sensing and means that bacteria behaviour can be controlled (Miller and Bassler, 2001). One could therefore imagine a mechanism leading to cell death at the level of a community of bacteria. For example, when the population reaches a certain threshold in terms of size and understands that it is time to stop growing.

Strategy 4: Design for dependency

Every living organism has specific minimal needs to survive. For instance, most plants need sunlight, water and some nutrients usually found in the soil, without one of these elements, they don't survive. The same applies to micro-organisms. This measure is similar to choosing the right organism and it is different as it concerns a design organism (instead of an existing one). The rationale is to design a dependency that makes survival only possible in designed conditions. This prohibits the modified organism to thrive where it is not wanted.

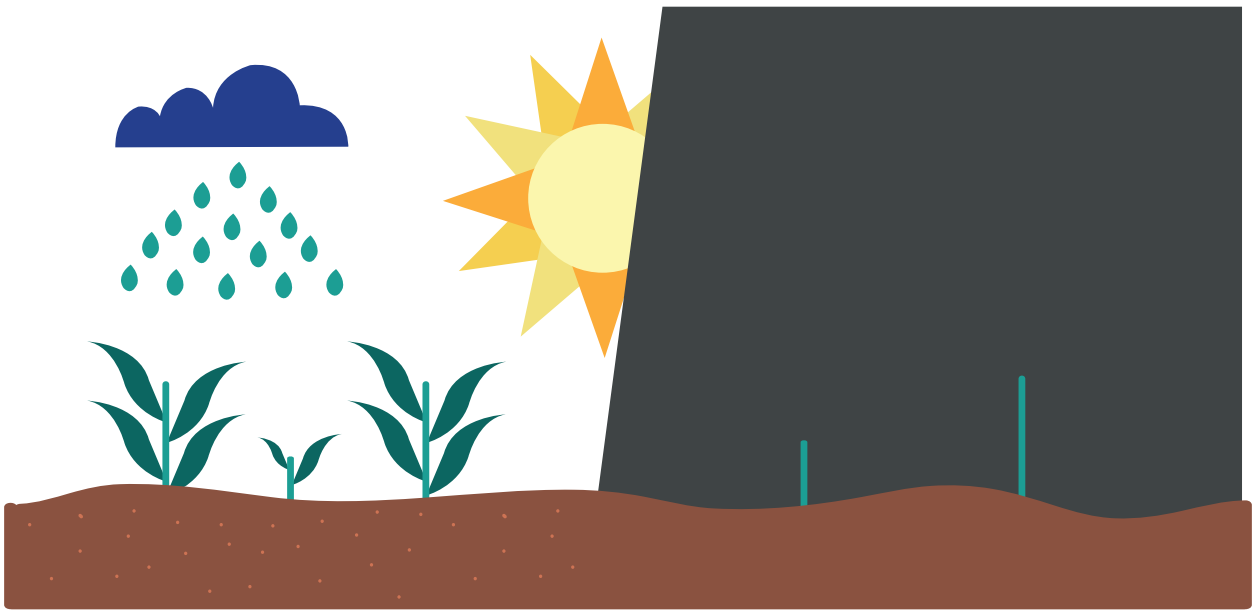

Illustration 4: Design for dependency

Measure: Auxotrophy

Living organisms need food to survive. The idea of auxotrophy in synthetic biology is to make use of what cells normally do with food and introduce a modification in the organism in order to control this basic process essential for survival. Imagine you could only eat if you are sitting at your kitchen table because your kitchen table has special properties that allow you to eat. You cannot however choose to eat on your couch because your couch lacks these special properties. This is what designing for auxotrophy does to a micro-organism, it creates a dependency.

In the same manner as for kill-switches, after escaping the laboratory, or the reactor, the micro-organism with an auxotrophy should not be able to survive because it will lack something essential for its growth outside of the designed environment. Different mechanisms allow to establish auxotrophy in micro-organisms (Moe-Behrens 2013). In literature, auxotrophy is argued to be a more robust strategy as it involves a loss of function (Wright et al., 2013), as opposed to the kill-switch which adds an undesirable function.

One possible worry with auxotrophy as a Safe-by-Design strategy is that cells might find other components in nature that could be similar enough to the one they are missing, or have spontaneous mutations that make the measure less reliable (Hirota et al., 2017). Recent work by Hirota and colleagues (2017) addresses this challenge by modifying a normal process of uptake of a needed compound for growth by E. coli, to a process using a similar compound rarely found in nature. Moreover, if it is found it will only work in insufficient quantities. Their research found no escape of the modified E. coli over 21 days with an escape frequency, i.e. the likelihood of the measure not working on all cells, of 1.94×10^{-13}, a much lower than the escape frequency reported for the Cryodeath kill switch described in the previous section.

Kill-switches and auxotrophy are both effective measures, but as explained their efficiency might still be limited. This can also be explained by our limited capacity to test their efficiency experimentally. A general safety principle calls for several barriers of containment in case one fails. Kill-switches and auxotrophy are often found in conjugation with each other and other strategies. For instance, systems integrate the kill-switch with other barriers for biocontainment like GeneGuard (Wright et al., 2015), or the SafeChassis project of Prof. Martins dos Santos at Wageningen University and Research. One thing is sure: one can only expect more developments in that direction.

Strategy 5: Design distance between the natural and the synthetic

In Chapter 1, we briefly introduced the reader to DNA as being the great unifier of all living organisms. All codons can be translated to specific peptides, regardless whether this process takes place in a bacteria or in a zebra. While this is a wonder of nature, it is also a cause for concern. Indeed, in Chapter 1, we also introduced risks of gene pool contamination, and horizontal gene transfer. These risks exist precisely because organisms can talk to each other thanks to these common base pairs making up the DNA. One strategy to address this is preventing this communication between organisms, it is sometimes also called semantic containment. There are three main measures within this rationale: orthogonality, xenobiology, and recoding. These measures do not exclude each other and each offer a multitude of options.

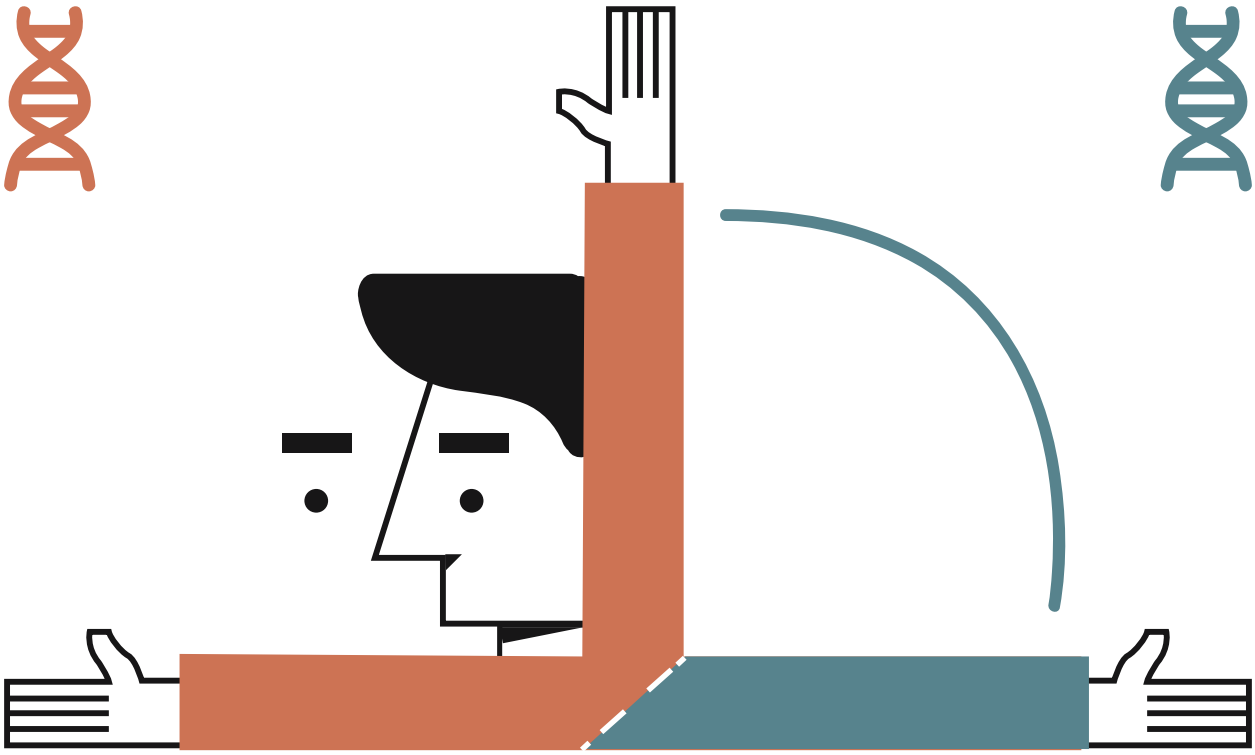

Illustration 5: Design distance between the natural and the synthetic

Measure 1: Orthogonality

In mathematics, orthogonality implies a perpendicular angle between two lines. In synthetic biology circuits, orthogonality means that the circuitry of the cell (that is usually prone to communication), can be controlled by another system that we know is definitely independent. This measure makes sure

that there is an independent control of the cell that will not be disturbed by mechanisms in the cell. Recent research shows that this can be achieved in many ways (Blount et al., 2012). Such a measure comes from the engineering view described in section 3.2.1 on Kill-Switches. Such measures increase the predictability of the behavior of cells (Endy, 2005).

Measure 2: Xenobiology

Xenobiology implies replacing known elements of life and cell machinery, which is based on DNA, carbon bonds, and specific sequences, for other base pairs of DNA, other cell machinery, other bonds, and other sequences. "Other" here means non-naturally occurring, or synthetic. In short, xenobiology means biology with foreign elements. In 2010, Schmidt introduced xenobioloy as the "ultimate biosafety tool" and speaks of a genetic firewall. Using xenobiology makes it very difficult, if not impossible for modified cells to communicate with other cells as they occur in nature, for instance by using XNA instead of DNA. In this paper, Schmidt already lays out design requirements for xenobiology.

While this might seem far-fetched to some, the next paragraph presents two recent results of applications of xenobiology found in literature. As the reader will notice, these examples are not exclusive to designing distance between the synthetic and the natural as a strategy. Indeed, they combine with the strategy of creating dependency.

Synthetic auxotrophy functions in the same way as metabolic auxotrophy, as described in the previous section. However, instead of removing the ability of a micro-organism to grow without a given compound that is natural, the organism cannot grow without a given compound that is not found in nature. Recent research (Mandell et al., 2015) achieve this with *non-standard amino acids*, or amino-acids that for the most part do not code for protein, so that are synthetic, and have to be supplemented to the micro-organisms. Another method for this is to create an organism that requires a specific compound for its cell machinery to work. Other recent research (Lopez and Anderson, 2015) realize this using a *different ligand* (a ligand is a small molecule that allows the binding of the DNA double helix) which has to be supplemented.

Measure 3: Recoding

Recoding the genetic code means to change the compositions of codons and the amino acids they code. Let us consider a fictitious example to understand this. Tryptophan is an amino acid coded by TGG. In a recoded genome, tryoptophan could perhaps be coded by CAA, along with all the other amino acids. It can be applied to any kind of nucleic acid XNA or DNA. Recoding could happen in at least two ways. Recoding in order to ***code for non-standard amino acids*** (Lajoie et al., 2013, Amiram et al., 2015), or a sort of ***cryptocode***, where the DNA stays the same, but the combination of base pairs that normally code for an amino acid would change. Such efforts also go in the direction of engineering the cell and recoding can also imply getting rid of genetic information deemed unnecessary (Ostrov et al., 2016).

Increasing the distance between the synthetic and the natural is therefore a rationale with very tangible strategies. Just like for previous strategies of self-destruction, questions remain. Dr. Markus Schmidt points out that one of the main questions is how much distance we should build between the synthetic and the natural to ensure that living systems, synthetic or not, will not adapt their components to finally communicate with each other.

Strategy 6: Sculpting evolution

One of the main features of biotechnology is that it deals with living organisms, and living organisms have the ability to reproduce. Through reproduction, traits are transmitted to future generations. A number of methods deal with controlling reproduction, e.g. in hybrid plants or the sterile insect technique. While these are not intended for safety, controlling the process of reproduction, i.e. what traits are passed on and to what extent, allows sculpting evolution. This contributes to safety by avoiding large changes in a population's genetic make-up which could have unintended effects on other species or ecosystem functions.

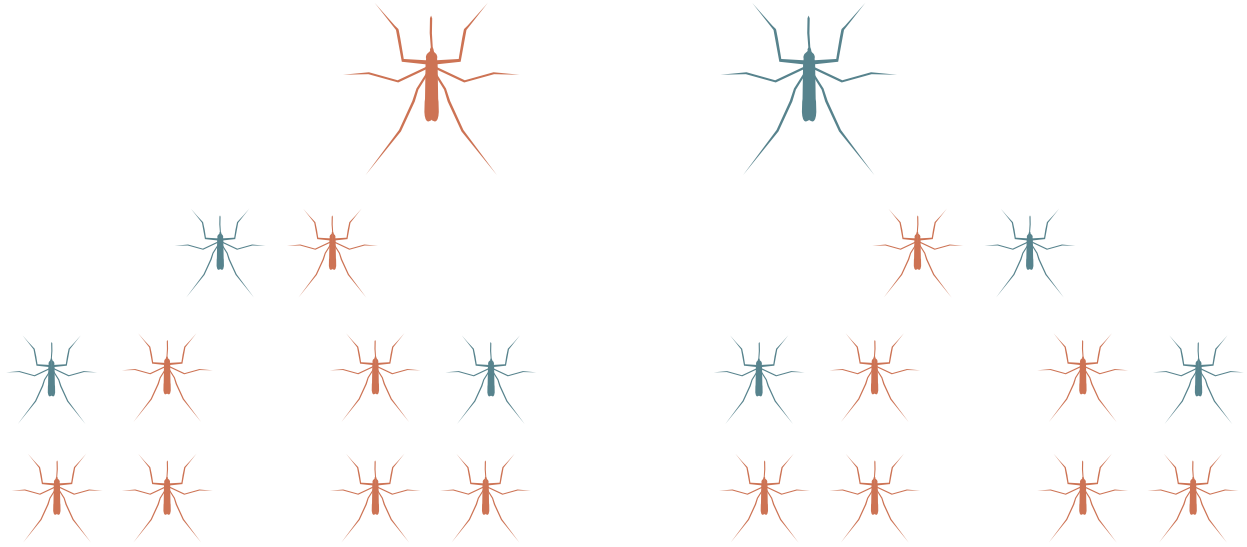

Illustration 6: Sculpting evolution

Hybrid plants are well known in the field of agriculture. You need both parents to make a vigorous offspring as these crops will not produce vigorous seeds by themselves. Recent literature suggests that we still have a lot to learn about the genetics of hybrid plants (Maheshwari and Barbash, 2011; Kim and Zhang, 2018). Another methods known to control what traits are passed on is the ***Sterile Insect Technique*** (SIT), which exists since the 1950s and aims at introducing sterile insects in a population as a mean of population control (Dunn and Follett, 2017). Both these methods are not made for the goal safety, but they can be seen as precursors of a strategy that aims to sculpt, or control the direction of evolution. A new method in biotechnology that controls what traits are passed on to future generations is the idea of gene drive, explained in Chapter 1. If traits passed on are particularly advantageous for the individuals in the population, such intervention in sculpting evolution could lead to invasive species (Noble et al., 2016).

Measure: Daisy Drives

A Safe-by-Design strategy is formulated by Kevin Esvelt and his team: daisy-drive (Noble et al., 2016). This measure creates a gene drive that is limited in time and space, and only uses a small population. In theory, this would allow making incremental changes to the disease carrying population without drastically changing the make-up of the population, and thereby avoiding tilting the ecosystem balance. Daisy drives therefore play a role as a Safe-by-Design measure in that they limit the potency of other

techniques such as gene drive by scaling down their impact. Daisy drives could be used to more safely sculpt the evolution of species by eradicating infectious diseases such as malaria, or Lyme disease.

Strategy 7: Control with external stimuli

Living organisms interact with their surroundings, so it is possible to engineer them to have certain reactions under certain conditions. In an earlier section the idea of quorum sensing was introduced, which means that bacteria can collectively modify their behavior. What else could control their behavior? In recent years, neuroscientists have discovered pioneered the field of optogenetics, whereby cell functions can be controlled by light (Pastrana, 2011). The field of optogenetics applied to biotechnology means that controlling the behavior of an organism at a distance is then quite literally possible by simply flipping a light switch. It is a measure that can apply to some of the other mentioned strategies because it controls behaviour.

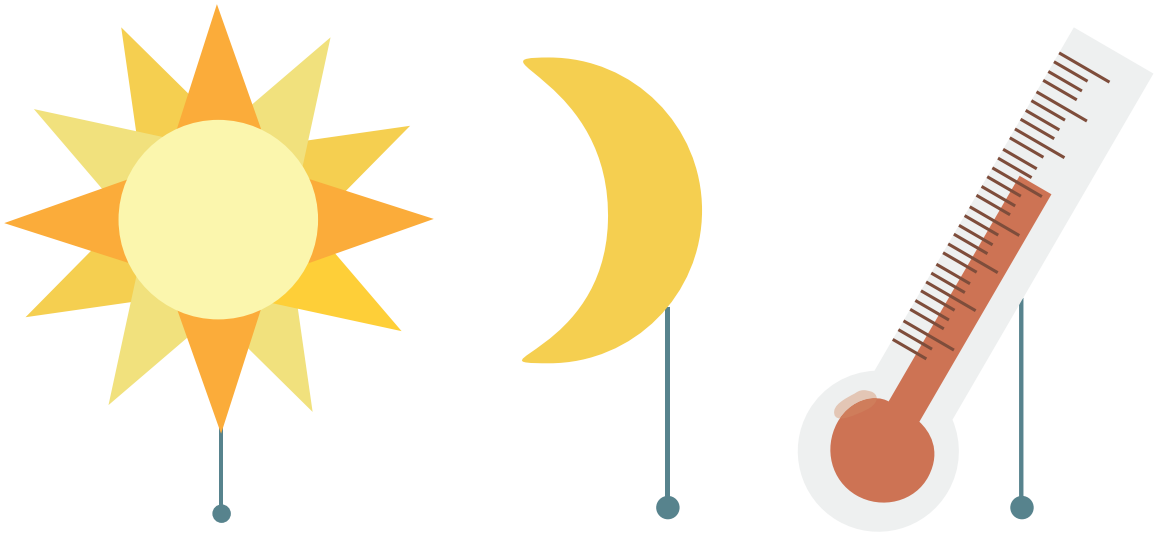

Illustration 7: Control with external stimuli

A possibility for controlling at a distance is for example, the optogenetic kill switch developed in 2016 by the Wageningen iGEM (WUR iGEM, 2016). Optogenetics can also serve to control cell reproduction (Polstein et al., 2017), or perhaps could be used in cell communities to control cell signalling (Kolar and Weber, 2017).

The possibility to use optogenetics as a design choice offers interesting options for biocontainment.

Yet, much is unknown about the efficacy and efficiency of particular applications in the field of biocontainment. Looking at other external stimuli is also interesting.

A recent review of iGEM strategies for Safe-by-Design or biocontainment mentions temperature as a way to control cell behavior (Whitford et al., 2018). Further research could explore what other stimuli exist for Safe-by-Design.

Strategy 8: Design warning mechanisms

To design warning mechanisms enhances safety by requiring immediate human action. Micro-organisms can be engineered to sense, smell, or recognize certain unwanted risky components and to emit a signal such as a change of colour. Unlike other strategies, this strategy requires human action to be taken to prevent potential hazards to happen or to minimize these hazards.

Illustration 8: Design a warning mechanism

In recent literature, biosensors have shown to have applications in multiple fields, from drug discovery to environmental monitoring, and imply a variety of different techniques or mechanisms (Vigneshvar et al.,2016). Bio-sensors are applications often developed in the iGEM competition. A successful example was the 2009 Cambridge university team's innovation called e.Chromi1[4]. The engineered machine could produce different colors reacting with different pollutants or diseases, thereby creating a quick and easy-to-use diagnostic tool. Another interesting example is the one of the Food Warden, a

4 | *http://thisisalive.com/e-chromi*

Figure 4: Summary of strategies and measures for Safe-by-Design

synthetic biology application that the Groningen iGEM team developed in 2012 (iGEM Groningen, 2012). By "smelling" rotten meat and being embedded in food packaging, the bio-sensor could indicate more accurately whether meat was safe to eat. The bio-sensor itself had Safe-by-Design measures like an auxotrophy and a kill-switch, as well as a very strong physical barrier though the pocket in which it was contained. This is yet another example of a multi-layered Safe-by-Design approach.

This chapter has highlighted some of the main strategies found in the literature. It is not exhaustive but gives the reader good pointers on where to look for more, and what to look for. In addition, certain strategies highlight that the idea of safety is not so straightforward. The next chapter outlines some of the challenges ahead for Safe-by-Design strategies in biotechnology.

4 Challenges of Safe-by-Design

In Chapter 1, the developments of biotechnology, their potential, the related new techniques, and their risks are introduced. In Chapter 2, the idea of Safe-by-Design, or designing for safety is explored in conceptual terms with examples from other technologies. Chapter 3 presents the potential of Safe-by-Design strategies in biotechnology. While many of these strategies are still rather in their infancy, studying their potential also raises several questions. This chapter presents five questions that emerged from discussing Safe-by-Design strategies:

How safe is safe enough?

How do we deal with the complexity of living systems?

What risks do we actually address with these strategies?

What are other strategies for realising safety?

What about evolution and Safe-by-Design?

4.1 How safe is safe enough?

In Chapter 3, the great variety of possible strategies and measures that could go towards doing Safe-by-Design in biotechnology are introduced. At several moments, in Chapter 3, examples are given where several of these measures are used in combination. This could be called a multi-layer approach, where several measures, or safeguards are added. This is desirable in case some of them fail to work. How many of these safeguards are needed to ensure safety? In other words, how safe is safe enough?

These questions raise two interesting issues. First, if we modify an organism to make it safer, do we still understand it? And second, who gets to decide what will be safe enough?

The first question is one of the main drivers behind of Prof. Martins dos Santos for creating a safe chassis[5], in other words, a model cell that would layer several safety measures from different strategies: recoding, auxotrophy and kill-switches. Their research project asks: if we take an organism we understand well, modify it to integrate several Safe-by-Design strategies, will it still be the same organism we know so well? This recalls of a classical thought experiment in philosophy, the ship of Theseus: after all parts of

5 | *https://www.nwo.nl/onderzoek-en-resultaten/programmas/onderzoeksprogramma+iw+biotechnologie+en+veiligheid/projecten/15814*

the ship have been replaced over time, is it still the same ship? While the ship of Theseus is a thought experiment that does not have a definite answer, the research at WUR is an empirical one that will answer this question for their specific model organism.

Experts say that an organism modified with Safe-by-Design strategies will be weaker than its wild cousin, because it is designed as such, and will therefore most likely not survive in the wild. The worry might therefore be what would happen with remaining strands of DNA after a micro-organism dies that might be taken up by other organisms and grant them unexpected properties. This is the topic of research mentioned in the expert interviews. This is another empirical question that is currently being investigated.

The second question is not an empirical one but rather a societal and political one: who decides when enough safety measures are in place? Given that different biotechnology applications will have different contexts, like a fuel producing bacteria, or a soil cleaning bacteria, perhaps the question of how safe is safe enough can be answered with the participation of the main stakeholders and lead to an understanding what safe enough means in these various contexts. Once a context-specific definition of safety standards is achieved, the role of Safe-by-Design strategies as described in Chapter 3 can be established. This is what the T-TRIPP project[6] aims to explore with its societal incubators.

Asking how safe is safe enough, requires empirical investigations, and societal and political debate. It is not about how many Safe-by-Design strategies are needed but rather what Safe-by-Design strategies make sense for a particular context and have a meaningful contribution to a larger safety approach.

6 | *https://www.nwo.nl/onderzoek-en-resultaten/programmas/onderzoeksprogramma+iw+biotechnologie+en+veiligheid/projecten/15809*

4.2 How do we deal with complexity of living systems?

Table 2: Safe-by-Design and the level of intervention

Strategy	Measure	Level of intervention
Choose the right organism	Not toxic	uni-cellular organism / organism
	Not pathogenic	uni-cellular organism / organism
	Environmental fit	uni-cellular organism / organism
Design physical barriers	Physical containment	uni-cellular organism
Design a self-destruct mechanism	Kill-switches	uni-cellular organism
Design a dependency	Auxotrophy	uni-cellular organism
Design distance between the synthetic and the natural	Orthogonality	uni-cellular organism
	Xenobiology	uni-cellular organism
	Recoding	uni-cellular organism
Sculpting evolution	Daisy drives	uni-cellular organism / organism
Control with external stimuli	Light	uni-cellular organism
	Temperature	uni-cellular organism
Design a warning mechanism	Biosensors	uni-cellular organism

Table 2 summarises the strategies identified for Safe-by-Design, their measures and at which levels these have an impact. It becomes very evident from this table that almost all of these measures can impact uni-cellular organisms. In a way, this limitation is to be expected at the early stages of research, where many of these strategies arise from an interest in understanding cell mechanisms and how to steer these through genetic engineering. Although born from scientific curiosity in understanding processes of life, the strategies can have applications for safety. This scientific curiosity is underlined by the diversity of options in each measure (for instance almost each laboratory will have its own kill-switch option). However, the overwhelming focus on uni-cellular organisms begs the question: how do we deal with the complexity of living systems? In this instance, complexity refers to both more complex organisms, and to organisms in a complex system.

When the organism is more complex, i.e. is not a unicellular organism, not all Safe-by-Design strategies can work in the same manner. For example, it seems difficult to imagine how the strategy of self-destruct would play out in a more complex organism. Whereas a strategy like choosing the right organism might easily translate to more complex organisms. Not all Safe-by-Design strategies apply to all types of organisms.

When the system is more complex, there are more effects to learn about and take into account. For instance, using gene drive to eradicate diseases carried by animals comes with challenges of potentially disturbing ecosystems. This problem has led to the formulation of daisy drive technology as an answer to those worries, as described in Chapter 3. Daisy drives still need testing as all other Safe-by-Design measures do. What are effects of other Safe-by-Design strategies on the ecosystem?

The current challenge therefore resides in formulating Safe-by-Design strategies that also cater to complex organisms, or complex environments. This challenge recalls an important distinction mentioned in Chapter 1: modified organisms are either contained or deliberately released. Do all strategies apply equally for both cases and lead to designing for safety? This question then leads us to the third challenge facing Safe-by-Design, on considering what potential hazards we actually address.

4.3 What potential hazards do we actually address?

Table 3: Potential risks addressed by Safe-by-Design strategies

Strategy	Measure	Risk scenarios addressed
Choose the right organism	Not toxic	Toxicity of modified organism
	Not pathogenic	Pathogenicity of modified organism
	Environmental fit	Invasiveness of modified organism
Design physical barriers	Physical containment	Spread of modified organism
Design a self-destruct mechanism	Kill-switches	Spread of modified organism
Design a dependency	Auxotrophy	Spread of modified organism
Design distance between the synthetic and the natural	Orthogonality	Horizontal gene transfer
	Xenobiology	Horizontal gene transfer
	Recoding	Horizontal gene transfer
Sculpting evolution	Daisy drives	Spread of modified organism and control for gene drive
Control with external stimuli	Light	Spread of modified organism
	Temperature	Spread of modified organism
Design a warning mechanism	Biosensors	Spread of modified organism

If the reader recalls the potential hazards mentioned in section 1.5 of this report, there were four main categories: persistence/invasiveness, or unintended effect on non-target organisms, gene pool contamination, horizontal gene transfer, and pathogenicity and toxicity to humans and animals. Almost all the strategies presented in Chapter 3 aim at preventing the spread of a modified organism. Yet, we need to learn about what happens when hazards materialize as risks.

One of the big challenges mentioned by experts was the need for experimental methods. For instance, Erik Joner speaks of how the effect of nano-particles is tested in soil with micro- and meso-cosms,

reproducing an ecosystem but within a controlled environment. Such tests not only allow to observe potential requirements but also allow to make efficient design choices. Erik Joner gives the example of one nanomaterial that could be made safer with Safe-by-Design choices but then becomes less efficient, which would require applying more of it and result in the same unwanted effects. Being able to experiment and see those trade-offs is important for making safe design choices that are both effective and efficient.

Learning about risks through experimentation, and specifying risk scenarios would allow making Safe-by-Design choices for safety more specific and could allow the formulation of new Safe-by-Design strategies.

4.4 What other strategies for Safety?

In Chapter 2, three aspects of fleshing out Safe-by-Design are presented: formulating the strategies for Safe-by-Design, facilitating the process through which these strategies were formulated, and the distribution of responsibility for safety. One aspect that emerges from Chapter 2 is the need for interdisciplinarity. In sections 4.1 , 4.2 and 4.3, the challenges presented focus on the strategies but also link these to how iterations can lead to better design choices. For instance, the need for empirical research, the need for societal involvement, and the need for experimental methods are all learning processes. With the knowledge acquired, one can return to the design and find ways of adapting the design accordingly. One challenge has not yet been addressed when it comes to Safe-by-Design. It has to do with the distribution of responsibility for safety and how different kinds of experts can contribute to safety.

In recent research, it was argued that instead of designing for safety, we should perhaps design for responsibility (van de Poel and Robaey, 2017). There are several reasons for this: current Safe-by-Design strategies imply that most of the responsibility for safety lies in the research and development phase. There are at least two problems why this alone might not be the most desirable approach: the efficiency of distributing responsibilities in the research and development phase and once the technology is out of the research and development phase there is a lack of means to deal with unanticipated risks.

First, overburdening those who make design choices might create a less efficient safety system.

Responsibility for safety should be distributed in a way that achieves its goal without overburdening stakeholders. It is questionable whether it is achievable to anticipate every safety aspect in advance, and design all risks out of the modified organism. What roles could other stakeholders play in achieving an efficient safety system?

This links to the second problem on dealing with unanticipated risks once the innovation is in use. To begin with, the SCENIHR report writes, "no single technology completely manages all biosafety risk" (2015, 9). Risk management principles like creating multiple fail-safes might not be enough. In addition, trying to design out all risks from the beginning ignores different types of uncertainties that may arise later. Indeed, once an innovation is in use, the designers are no longer at the frontline of safety, but other actors, like users, should also have the possibility to act for safety (Robaey, 2016). These actors have a different kind of expertise.

While Safe-by-Design strategies offer promising avenues to achieve safety, Safe-by-Design as a concept can consist of more than the types of strategies listed in Chapter 3. Tim Trevan argues for a safety culture in biotechnology, inspired from lessons learned in other engineering fields and even the health sector (2015). Biotechnology is an enabling technology with applications in agriculture, food, environment, medicine, energy, materials, and probably many other fields of application. Not only do these applications have different safety challenges (e.g. food safety, vs environmental safety), but also can these applications be situated in a contained environment or deliberately released.
Broadening the scope of Safe-by-Design seems necessary for the formulation of strategies that deal with defining what is needed for safety (section 4.1), dealing with the complexity of life (section 4.2), and addressing the specificity context of risk (section 4.3). To do this, the scope of involved stakeholders must be broadened. This in turn can help giving Safe-by-Design a meaningful place in risk management of biotechnology.

4.5 What about evolution?

Last, but not least, a major challenge of Safe-by-Design in biotechnology is that living organisms are prone to mutations, and forces of evolution, like all living organisms.

If a trait is not useful to an organism, it has a high likelihood of not being passed on to its offspring. In addition there are random mutations that occur in evolution. This is what is observed with many strategies within the self-destruct rationale, where the Safe-by-Design measure is not being retained after a certain about of time. In general, we lack experimental methods to verify the efficacy over long term and less controlled environments to test these rationales that go against the survival of the organism. Should we continue to pursue them? Or should we rather focus on more evolutionarily stable strategies?

Evolution is the opposite of design as it does not follow a plan with carefully considered choices (Calvert, 2014). The strategies described in Chapter 3 all aim at making carefully considered choices. Organisms always look to reproduce and grow. The reflection that Jane Calvert formulates was echoed in some of the expert interviews. This is observation is also found in an ethnography of synthetic biology, where a trend in synthetic biology to simplify nature and putting research efforts into modifying living organisms so that they behave like predictive models, instead of making models that would better predict the behavior of organisms is described (Roosth, 2016). Perhaps, instead of enhancing controllability in an engineering sense, making use of evolution as a design principle could lead to new rationales, or paradigms that could also enhance safety.

This chapter highlights some of the main challenges ahead for Safe-by-Design. These challenges call for empirical investigations, political and societal debate , and participation of various stakeholders in the definition of safety design options. How do we take it from there?

5 What's next for Safe-by-Design in biotechnology?

Safe-by-Design in biotechnology is a concept that has not yet been formalized in biotechnology. This report investigates the meaning concept meant in other fields (Chapter 2), the available strategies in biotechnology that can be understood as Safe-by-Design (Chapter 3), and the challenges of Safe-by-Design (Chapter 4). This analysis allows formulating three main points of attention for the future of Safe-by-Design in biotechnology. First, there is a need for more empirical research on the potential hazards of biotechnology and on Safe-by-Design strategies themselves. Second, there is a need to open up the notion of safety and link the Safe-by-Design strategies to a process that allows understanding their role. Third, there needs to be a steering force giving direction to this broad field of safety research.

5.1 Need for more research

The need for research is two-fold. We need to learn more about hazards and risks and we need to learn more about the long term efficiency and impacts of Safe-by-Design strategies. Learning more about risks is essential to understanding how to prevent them, or manage them. Learning implies specifying risks to a particular biotechnology application and its context. Learning also implies having accurate experimental methods.

The Safe-by-Design strategies described in Chapter 3 focus on biocontainment, or the risk of spread of modified organisms. There are more specific lines of research suggested summarizing four areas of risk research for modified organisms as: 1) the physiological differences between natural and synthetic organisms and interactions with the environment, 2) the impact of escaped organisms on ecosystems, 3) the likelihood of adaptation and evolution of modified organisms in the wild, and 4) the likelihood of gene transfer from modified to wild organisms (Dana et al., 2012). These points of research can be even further specified to specific applications and cases. While this research is gaining attention, it could be more actively integrated with the formulation of Safe-by-Design strategies and measures.

Environmental scientist Todd Kuiken explains that questions of risk assessment for modified organisms are not radically different to other risk assessments; it's about understanding impacts on the environment.

Understanding impacts allow preventing them, and managing them.Once risks are further specified and strategies are formulated, further research is needed to understand how well these strategies work. The SCENIHR report (2015) reports that the scale, the speed and the increasing variety of applications of modified organisms will make testing strategies on all of them challenging. Testing them would mean understanding their efficacy, efficiency and interactions.

More methods are needed to test escape frequencies (or how well a Safe-by-Design measure works), numbers of generations that retain the Safe-by-Design measure, and lag phases (or how long it takes for the Safe-by-Design measure to work). Currently, this research has only been realised in lab settings. Biosafety expert Markus Schmidt further underlines the need to have testing methods that allow setting benchmarks for required escape frequencies, or lag phases. Having benchmarks provides a mean to understand not only how well a Safe-by-Design measure works but also to understand how well it ***should*** work. Where do we set the limit on escape frequencies, generations retaining the Safe-by-Design measure and lag phase?

5.2 Opening up the notion of safety

Opening up the notion of safety has two main implications. First, it implies that what safety means should involve stakeholder perception of safety, as well as an interdisciplinary approach. Second, it means that stakeholders other than designers of modified organisms can play a role in achieving safety.

Safety is an important public value (InSites Consulting, 2017), meaning that society sees it as a common goal. Safety as a value is inextricably linked to public trust (Pauwels, 2013). In previous research about Safe-by-Design, stakeholders were found to identify many different safety issues that were linked not only to design, but also to accidents, or to errors in use[7] (Robaey et al., 2017). When formulating Safe-by-Design strategies, stakeholder involvement is crucial to understanding the full meaning of safety. Doing this in addition of empirical research will allow formulating Safe-by-Design strategies that have a broader scope than biocontainment. Stakeholder involvement can also help deciding whether these strategies play a useful role for safety. If so it can help to decide how many of these strategies should be used, and under what conditions they should be used to achieve safety.

Opening up the notion of safety is not only about stakeholder engagement, it is also about understanding

7 | Speaking of misuse reminds of the literature on bio-security, which is beyond the scope of this report but also relevant for the discussion on Safe-by-Design. Moreover, there are issues of accidental misuse that might or might not be relevant for the discussions of Safe-by-Design.

safety from different disciplinary points of view. Indeed different disciplines might identify different types of risks and might propose different safety measures. Opening up the notion of safety means inviting stakeholders and other experts into that process, and perhaps sharing responsibility for safety with them.

Thinking of safety more broadly can be organized through a process. Such a process can include clear points where certain information has to be known and where decisions have to be taken. It can also include iterations back to the design table to change the design. At each step of the process, stakeholders and other experts can be involved. For instance, risk assessor Esther Kok and her colleagues at RIKILT, have developed an approach called Safe-by-Strategy. This approach points to several stages of assessment during the project proposal, research and development phase, developed for a series of projects that formed part of the strategic plan on 'synthetic biology' of Wageningen University and Research, that is coordinated by professors Vitor Martins dos Santos and Dirk Bosch. At the different stages, specific safety issues are assessed in relation to current or subsequent stages of development, as well as how these safety issues can be investigated as part of the ongoing project. If sufficient data are available to conclude on the respective safety issues, the innovation may proceed to the next step. Within the strategic plan, a first, largely theoretic, start has been made with the application of this safe-by-strategy approach. Further work should be done in this direction for other fields in biotechnology.

5.3 Need for a steering force for Safe-by-Design research

Much research remains to be done to learn about Safe-by-Design. Also developing a process involving stakeholders and other experts, and allowing for iteration in the design will take time. In other words, the next steps of Safe-by-Design are costly in research funding, research attention and research time. There is a need for a steering force for Safe-by-Design research efforts.

Recently, members of the biosafety community have called for standardization in biotechnology (De Lorenzo and Schmidt, 2018). They argue that standardisation in biotechnology will allow better exchange of information and better risk assessments, among other things. Here, standards refers to standard biological parts, or circuits. Such a call for standardisation can give direction to the research being done at the moment. In addition De Lorenzo and Schmidt call for an active European involvement in the discussion of standards. Standards can also refer to safety standards. Huib de Vriend underlines

that it is important to know who decides how safety standards are defined and how these ought to be decided upon. Both empirical, societal, and political decisions on the matter of setting standards should be the results of a transparent discussion, and should happen in a manner that integrates all aspects.

To conclude this report, the need for a steering force for Safe-by-Design research in biotechnology is perhaps the most important message to take home. While a lot of research is being done, the available research is still in its infancy. Most (not all) of it stems from scientific curiosity rather than practical problems. In addition, if these measures are to be used, they need to be tested, they need to be focused on a problem and in a given context. Last but not least, to understand the place of these measures in dealing with safety, public consultations are necessary. This is why concerted research efforts and defined priorities are necessary.

This last section has summarized the main points of interest and focus of this report. What does the future hold for Safe-by-Design in biotechnology? This is something experts in biotechnology and related disciplines, as well as industry leaders and other stakeholders, together with governments, will have to define. Hopefully, this report will have provided a useful exploration in the emerging discourse of Safe-by-Design. It might contribute to future discussions about Safe-by-Design and it might even lead to outside-the-box design ideas for Safe-by-Design.

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Annex I – List of experts interviewed

1 Dr. Cécile van der Vlugt, National Institute for Public Health and the Environment (RIVM), The Netherlands
2 Dr. Christian Fleck, ETH Zurich, Switzerland
3 Dr. Erik Joner, Norwegian Institute of Bioeconomy Research, Norway
4 Dr. Esther Kok, RIKILT, Wageningen University and Research, The Netherlands
5 Dr. Huib de Vriend, LIS Consult, The Netherlands
6 Prof. Klaas Hellingwerf, University of Amsterdam/Photanol B.V., The Netherlands
7 Prof. Mark van Loosdrecht, Delft University of Technology, The Netherlands
8 Dr. Markus Schmidt, BioFaction, Austria
9 Dr. Todd Kuiken, Senior Research Scholar at the Genetic Engineering & Society Center at North Carolina State University, United States of America
10 Prof. Vitor Martins dos Santos, Wageningen University and Research, The Netherlands

Compiled definitions taken from different sources

Term	Definition
Bacterial strain***	Classification for bacteria, group of bacteria with genetic similarity.
BioBricks™ **	BioBricks™ is a standard for interchangable parts consisting of a DNA sequence, developed with a view to building biological systems in living cells.
Cell*	Cells are the basic building blocks of all living things. They provide structure for the body, take in nutrients from food, convert those nutrients into energy, and carry out specialized functions. Cells also contain [DNA] and can make copies of themselves.
Chromosome*	In the nucleus of each cell, the DNA molecule is packaged into thread-like structures called chromosomes.
Codon*	Translation, the second step in getting from a gene to a protein, takes place in the cytoplasm. The mRNA interacts with a specialized complex called a ribosome, which "reads" the sequence of mRNA bases. Each sequence of three bases, called a codon, usually codes for one particular amino acid.
DNA*	DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms.
Evolution*	Evolution is the process by which populations of organisms change their DNA over generations.
Gene*	A gene is the basic physical and functional unit of heredity. Genes are made up of DNA. Some genes act as instructions to make molecules called proteins.
Genome*	A genome is an organism's complete set of DNA, including all of its genes. Each genome contains all of the information needed to build and maintain that organism.
Mutation*	A gene mutation is a permanent alteration in the DNA sequence that makes up a gene, such that the sequence differs from what is found in most [other individuals].
RNA*	During the process of transcription, the information stored in a gene's DNA is transferred to a similar molecule called RNA (ribonucleic acid) in the cell nucleus. Both RNA and DNA are made up of a chain of nucleotide bases, but they have slightly different chemical properties. The type of RNA that contains the information for making a protein is called messenger RNA (mRNA) because it carries the information, or message, from the DNA out of the nucleus into the cytoplasm.
Toxin*	Internal substances formed within cells.
Transcription*	During the process of transcription, the information stored in a gene's DNA is transferred to a similar molecule called RNA (ribonucleic acid) in the cell nucleus.
Translation*	Translation, the second step after transcription in getting from a gene to a protein.

*(Sources: *US National Library of Medecine, 2018; **iGEM Registry of Standard Biological Parts, 2018; *** Baron, 1996)*