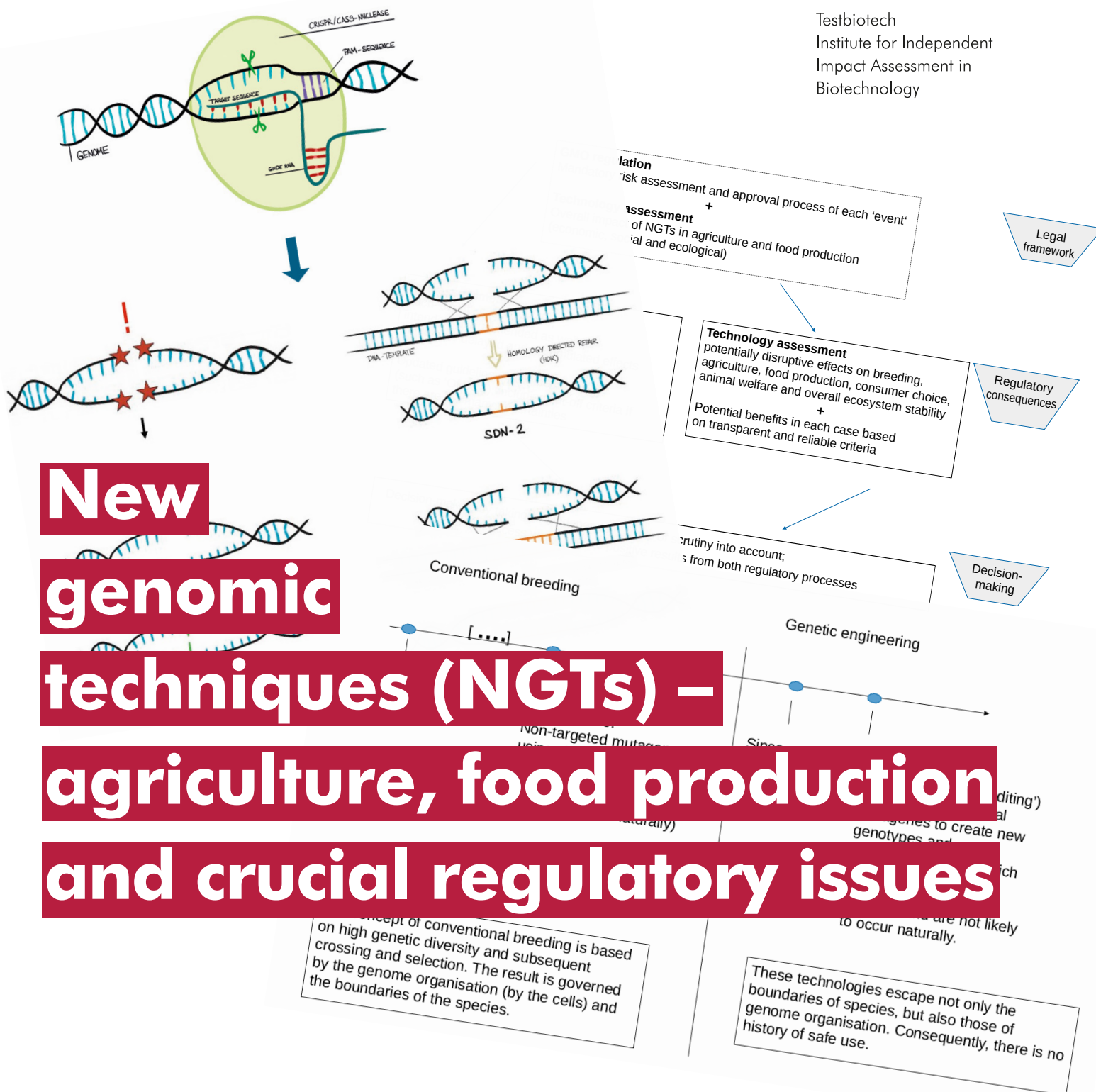


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Institute for Independent
Impact Assessment in
Biotechnology



New genomic techniques (NGTs): agriculture, food production and crucial regulatory issues

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Commissioned by and written for
Verbraucherzentrale Bundesverband (vzbv)



Supported by:



Federal Ministry
for the Environment, Nature Conservation,
Nuclear Safety and Consumer Protection

Grafics: Testbiotech / Timo Zett

Final Layout & Title: Claudia Radig-Willy

based on a decision of
the German Bundestag

Special thanks to

Astrid Österreicher, Nina Valenzuela, Andreas Bauer-Panskus and Matthias Juhas for their input.

Also thanks to the 'Project for Genetic Engineering and the Environment', their website was an important source of information for this report – <https://fachstelle-gentechnik-umwelt.de/en/home>

Imprint

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Abstract

This report was compiled to answer questions raised by the Federation of German Consumer Organisations (Verbraucherzentrale Bundesverband, vzbv) in regard to EU regulation of new genomic techniques (NGTs, also known as new genetic engineering or genome editing). For this purpose, it provides an overview of several aspects of NGTs which are especially relevant in the context of agriculture and food production.

The questions posed by vzbv arose from EU Commission initiatives to change the current EU GMO regulation. vzbv's most important (overarching) question is: *"What are the crucial requirements for an ideal regulation of NGTs from the perspective of the consumers and the protection goals regarding health, the environment and animal welfare?"*

Details of the technical characteristics as well as possible NGT applications in agriculture and food production are provided in the report, including several examples. It further examines risks and hazards in regard to plants, animals and microorganisms. Finally, it includes an overview of requirements for the regulation of NGTs and the assessment of associated risks.

The report presents the following conclusions and answers:

EU regulation of NGTs should prevent:

- uncontrolled marketing or releases of NGT-GMOs into the environment;
- damage to biological diversity, ecosystems and agriculture;
- health hazards from being introduced unnoticed into the food system where they might accumulate;
- data needed for risk assessment by independent experts being treated as confidential business information;
- contamination of organic and other food or seed production systems that exclude the use of genetically engineered organisms.

EU regulation of NGTs must ensure:

- a case-by-case risk assessment and an approval process for each NGT 'event', including taking accumulated effects into account;
- the further development of data requirements, guidelines and methods of risk assessment to achieve the highest safety standards, including cut-off criteria in cases where uncertainty is too great;
- the availability of information to track and trace the NGT-GMOs and food products derived thereof;
- measures are in place to prevent the uncontrolled spread of NGT-GMOs in the environment;
- consumer choice and coexistence with organic and GE free food production;
- animal welfare is fully respected at all stages of the NGT processes;
- prospective and comprehensive TA is carried out before NGTs are brought to market.

Consequently, all NGT-GMOs need to undergo a mandatory approval process before being released into the environment or brought to market. Risk assessment should (as currently requested by EU legislation) aim to identify the intended and unintended changes resulting from the technical processes of genetic engineering and should evaluate their potential to cause adverse effects on human health and the environment. The differences between conventional breeding and NGTs can be easily overlooked, but can have serious consequences. In this context, direct and indirect effects which may be immediate, delayed or cumulative have to be taken into account.

Furthermore, a comprehensive and prospective technology assessment is essential prior to use in an agricultural setting in order to address systemic risks to biodiversity, socio-economic impacts and effects in regard to sustainability. There are no mechanisms in place and no data available to distinguish 'empty promises' from 'real benefits'. In summary, NGTs, e.g. CRISPR/Cas, have huge potential to alter the genome but this potential

does not easily translate into real benefits. Technology assessment should be carried out in accordance with the precautionary principle and, at the same time, evaluate the actual need to apply the technology and also to consider alternatives that could be made available. The single overarching principle should be to generally restrict releases of NGT-GMOs into the environment to avoid, e.g. passing potential tipping points leading to irreversible damage in ecosystems.

Kurzfassung

Dieses Gutachten dient dazu, Fragen des Verbraucherzentrale Bundesverbandes (vzbv) im Zusammenhang mit der EU-Regulierung neuer genomischer Techniken (NGT, auch Neue Gentechnik, Genome Editing) zu beantworten. Zu diesem Zweck gibt das Gutachten einen Überblick zu verschiedenen Aspekten die insbesondere im Bezug auf Landwirtschaft und Lebensmittelproduktion wichtig sind.

Die Fragen ergeben sich vor dem Hintergrund der Initiativen der EU-Kommission, die bestehende Gentechnik-Gesetzgebung zu verändern. In diesem Zusammenhang stellt der vzbv die (übergreifende) Frage: „*Welches sind die zentralen Anforderungen an eine aus Verbraucher-, Umwelt- und Tierschutzsicht optimale Regulierung neuer gentechnischer Verfahren?*“

Das Gutachten erläutert relevante Details in Bezug auf die technischen Eigenschaften der NGTs und mögliche Anwendungen in der Landwirtschaft und Lebensmittelproduktion, die an mehreren Beispielen illustriert werden. Die Risiken werden im Hinblick auf Pflanzen, Tiere und Mikroorganismen diskutiert. Schließlich wird ein Überblick über notwendige Anforderungen an die Regulierung von NGTs und die Bewertung der damit verbundenen Risiken gegeben.

Kurz zusammengefasst können aus diesem Gutachten, die folgenden Antworten abgeleitet werden:

Die EU-Regulierung von NGTs muss verhindern, dass

- gentechnisch veränderte (NGT-) Organismen (NGT-GVOs) auf unkontrollierte Art und Weise freigesetzt oder vermarktet werden;
- Schäden an der biologischen Vielfalt, den Ökosystemen und der Landwirtschaft eintreten;
- sich gesundheitliche Gefahren unbemerkt in die Lebensmittelerzeugung einschleichen und akkumulieren können;
- Daten, die für die Risikobewertung benötigt werden, als Geschäftsgeheimnis klassifiziert werden;
- ökologische und andere gentechnikfreie Produktionssysteme für gentechnikfreie Lebensmittel, Saatgut und Landwirtschaft kontaminiert werden.

Die EU-Regulierung muss zudem sicherstellen, dass

- jeder NGT-GVO einen Zulassungsprozess inkl. Risikoprüfung durchläuft und auch akkumulierte Effekte berücksichtigt werden;
- die Anforderungen an relevante Daten, Richtlinien und Methoden der Risikobewertung weiterentwickelt werden, um höchste Sicherheitsstandards zu gewährleisten; dazu gehören auch Kriterien zum Abbruch des Zulassungsprozesses (Cut-Off-Kriterien), falls zu viele Unsicherheiten bestehen;
- die notwendigen Informationen vorhanden sind, um die NGT-GVOs und aus ihnen gewonnene Lebensmittel zu detektieren und zu verfolgen;

- › Maßnahmen gegen eine unkontrollierte Ausbreitung von NGT-GVOs in der Umwelt ergriffen werden;
- › die Wahlfreiheit für die Verbraucher:innen und die Koexistenz mit ökologischer und gentechnikfreier Lebensmittelproduktion gewährleistet ist;
- › der Tierschutz auf allen Stufen des Einsatzes von NGT-Verfahren vollumfänglich respektiert wird;
- › eine umfassende und vorausschauende Technikfolgenabschätzung durchgeführt wird, bevor NGTs zugelassen werden.

Daraus folgt, dass alle NGT-GVOs einer verpflichtenden Zulassungsprüfung unterzogen werden müssen, bevor sie freigesetzt und/oder vermarktet werden können. Die Risikobewertung muss (wie derzeit von der EU-Gesetzgebung verlangt) alle beabsichtigten und unbeabsichtigten genetischen Veränderungen identifizieren, die durch den Prozess der gentechnischen Verfahren verursacht werden und diese im Hinblick auf mögliche Schäden für Mensch und Umwelt bewerten. Die spezifischen Unterschiede zwischen NGTs und natürlichen Prozessen (bzw. konventionellen Verfahren zur Züchtung) sind leicht zu übersehen, können aber schwerwiegende Konsequenzen haben. Dabei müssen direkte und indirekte Effekte berücksichtigt werden, die unmittelbar, verzögert oder kumulativ sein können.

Zudem muss vor dem Einsatz von NGTs in der Landwirtschaft eine umfassende und vorausschauende Technikfolgenabschätzung durchgeführt werden, um sozio-ökonomische Auswirkungen, Effekte auf die Nachhaltigkeit und mögliche Kippunkte für die Ökosysteme zu bewerten. Es bedarf Leitlinien und Kriterien, um zwischen ‚tatsächlichen Vorteilen‘ und ‚leere Versprechungen‘ zu unterscheiden. Die Verfahren der NGTs haben zwar ein großes Potential für genetische Veränderungen, aber es ist nicht einfach, dieses Potential in tatsächliche Vorteile umzusetzen. Die Technikfolgenabschätzung muss in Übereinstimmung mit dem Vorsorgeprinzip durchgeführt und dabei auch der tatsächliche Bedarf für NGTs sowie mögliche Alternativen im Detail geprüft werden. Dabei sollte es ein Ziel sein, die Freisetzungen von NGT-Organismen möglichst zu begrenzen, um bspw. mögliche Kippunkte zu vermeiden, die zu irreversiblen Schäden an den Ökosystemen führen.

Summary

This report was compiled to answer questions raised by the Federation of German Consumer Organizations (Verbraucherzentrale Bundesverband, vzbv) in regard to EU regulation of new genomic techniques (NGTs, also known as new genetic engineering or genome editing). For this purpose, it provides an overview of several aspects of NGTs which are especially relevant in the context of agriculture and food production.

Details of the technical characteristics as well as possible NGT applications in agriculture and food production are provided in the report, including several examples. It further examines risks and hazards in regard to plants, animals and microorganisms. Finally, it includes an overview of requirements for the regulation of NGTs and the assessment of associated risks.

What is new about NGTs?

NGTs can be used to achieve genomic changes extending far beyond what is known from conventional breeding. Compared to methods of conventional breeding (including random mutagenesis), NGTs can overcome the boundaries of natural genome organization resulting from evolution. In particular, CRISPR/Cas gene scissors make it possible to genetically alter the genome to a much greater extent compared to previous breeding methods.

The greater accessibility of the genome enables

- pervasive changes in the biological characteristics of organisms even without the insertion of additional genes;
- the creation of new genotypes by overriding the natural genome organization, such as repair mechanisms (or other protective factors such as gene duplications), thus generating new genotypes that extend far beyond what can be achieved by conventional breeding;
- more extreme versions of already known traits or the generation of new traits which come with 'trade-offs' (side effects).

In addition, the technical potential of tools such as CRISPR/Cas is associated with a high potential for unintended genetic changes that are unlikely to occur naturally.

Several factors can impact the outcome of NGT processes in regard to intended and unintended effects, e.g. species, trait, target genes (their site, their function, their number, similarities with other genes), the gene scissors themselves (or other tools used) and the process of introducing the gene scissors (or other NGT tools) into the cells.

Potential applications in agriculture and food production

The range of species accessible to NGTs extends far beyond applications of previously used genetic engineering techniques, although their effectiveness may differ from case to case. It includes a wide range of food plant species and animals, and also non-domesticated species, such as trees and other plants, insects, vertebrates and microorganisms, thus covering all domains of life. Many of the species targeted by NGT-applications also have the potential to persist and spread over longer periods of time.

Examples of relevant applications were taken from several databases and include plants, animals, mushrooms and microorganisms. However, either no or only limited conclusions can be drawn from these data to predict which of the NGT-GMOs will eventually be brought to market and when.

Plants and mushrooms

Desired traits in plants are e.g. changes in plant composition, stress tolerance (biotic and abiotic), yield, herbicide resistance and improved storage. We include an overview of plants that are already being marketed (tomatoes with changed nutritional composition, going along with health claims, and soybeans with altered oil composition). Further examples include mushrooms with delayed browning, herbicide-resistant maize, camelina with changes in oil composition, *de novo* domesticated tomatoes and wheat (several traits). In each case, we also discuss the intended and unintended effects.

Animals

Applications in animals used for food are related to meat quality and yield as well as adaptation to climate change or animal husbandry. We include an overview of animals already allowed for marketing (two NGT fishes, involving two species, with altered growth in Japan and one NGT 'slick' cattle in the US). Further examples include hornless cattle and hens which supposedly have no male offspring. In each case, we also discuss the intended and unintended effects.

Microorganisms and viruses

Applications of NGTs may also involve microorganisms, such as bacteria, archaea, fungi and yeast, and in some cases, viruses. The aims in these cases would, for example, be related to soil quality, the microbiome of plants and animals, pesticides and paratransgenesis (impacting the biological characteristics of plants or animals by changing their associated microbiota).

What are the risks associated with NGT-GMOs?

Both intended and unintended genetic changes arising from applications of NGTs often are vastly different compared to changes seen in conventional breeding (including random mutagenesis). In some cases, the differences between naturally occurring processes (or conventional breeding) and NGTs may be easily overlooked, but nevertheless can have serious consequences for health and the environment. The report gives an overview of the risks caused either directly or indirectly by the intended traits as well as by the unintended genetic changes inherent to the processes of NGTs. There is some evidence that cumulative risks have to be taken into account, as these may lead to new dimensions of hazards.

(1) Risks caused unintentionally by the intended traits in plants and animals

As exemplified in camelina (with changed oil composition), wheat (e.g. for reducing gluten in baked products) and fish (sea bream and pufferfish with increased weight gain), the traits resulting from NGT can cause extreme variants of biological characteristics as well as new traits which are unlikely to be achieved with conventional breeding. The depth of the intervention may, from case to case, unavoidably lead to 'trade off' responses (metabolic side effects) in the organisms that are associated with unintended biological effects, e.g. weakening the tolerance of NGT plants to biotic or abiotic stressors or creating animal welfare issues.

These direct or indirect effects are the result of interactions in the complex networks of genes, proteins and other biologically active molecules. Such unintended metabolic and physiological effects can still emerge even in cases where the genetic intervention is targeted and precise.

In summary, effects associated with the intended traits may, for example, have serious adverse impacts on the environment, plant or animal health, agricultural yield, pesticide use and food safety. If released into the environment, the interactions with other NGT-GMOs and with the environment, including pests, pathogens, climatic conditions, etc. add further complexity to these risk scenarios.

(2) Risks linked to unintended genetic changes in plants and animals

As is the case with intended traits, unintended effects can also cause patterns of genetic change that go far beyond what can be achieved with conventional breeding, and thus result in specific risks. Effects include off-target DNA cleavage, repetitive unit deletion, indels of various sizes, larger structural changes in the targeted genomic region and unintended insertion of transgenes. If these unintended effects are overlooked, they may quickly spread within large populations. For example, NGTs were successfully applied in cattle to generate a 'hornless' trait. However, the unintended insertion of additional genes from bacteria, including genes that confer resistance to antibiotics, was discovered some years later. Luckily these animals had not been used for commercial breeding, otherwise these undesirable genetic conditions could have been spread rapidly and widely within the populations. The same problem may occur if seeds used for further propagation and breeding contain hazardous genetic alterations which remain undetected and accumulate over a longer period of time.

In this context, it has to be taken into account that NGT is a multi-step process with inherent and specific risks independent of the desired traits. For example, in plants, NGTs such as CRISPR/Cas typically make use of the older genetic engineering ('Old GE') and non-targeted methods to deliver the DNA coding for the nuclease into the cells. Thus, in most cases, the first step of the CRISPR/Cas application results in a transgenic plant which may have a wide range of unintended genetic changes unlikely to emerge from conventional breeding. Conventional breeding is only used at the end of the multistep process to remove the transgenic elements from the plant genome (segregation breeding). However, without adequate risk assessment standards the unintended genetic changes may remain undetected in the genome, and thus spread and accumulate both rapidly and widely in populations.

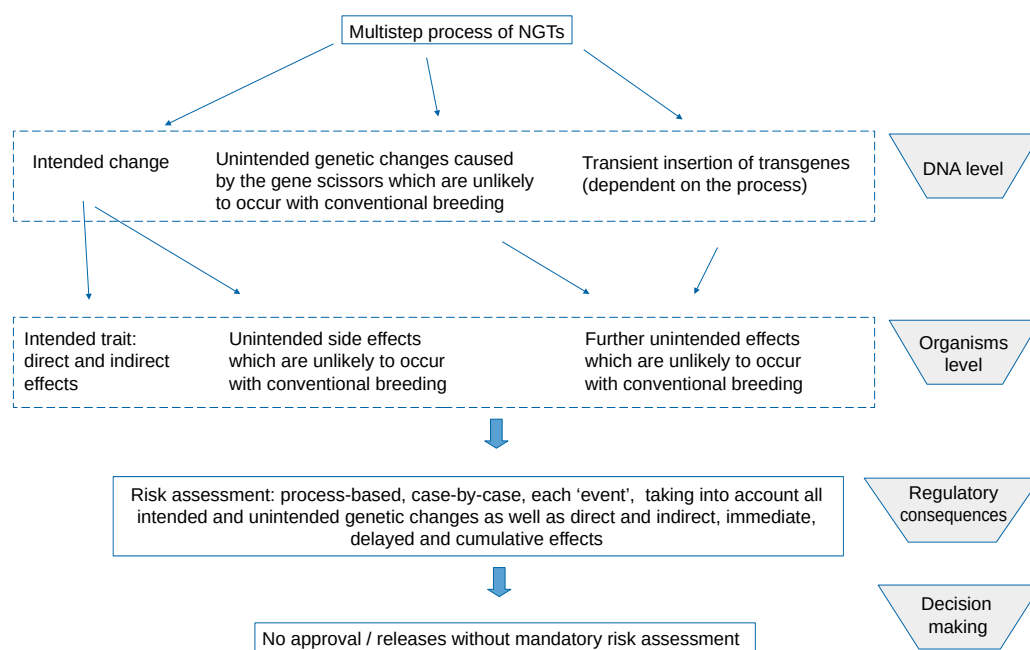


Figure 1: Current regulation of NGTs: Intended traits, unintended side effects and unintended genetic changes triggering need for risk assessment

In summary, at each stage of the process - including (i) insertion of the DNA contained in the gene scissors into the cells, (ii) target gene recognition and cleavage and (iii) cellular repair of the genes - specific unintended alterations can occur that are associated with risks (see Figure 1).

(3) Risks linked to microorganisms

Once they are released, genetically engineered microorganisms can often survive and persist in the receiving environment, and thus invade new environments leading to multiple interactions with other organisms. Even microorganisms that are only meant for contained use can, in certain circumstances, spread in the environment: experience gathered from genetically engineered microorganisms used in food production shows that these applications may result in large-scale contamination of food and feed, for example, with genetically engineered bacteria or bacterial DNA.

In general, many microorganisms are closely associated with species from other domains (plants or animals) that are considered to be their 'hosts'. The microbiome of plants, insects, vertebrates and also humans is made up of specific combinations of microorganisms. If NGT microorganisms are released, they can survive and colonize in the microbiomes, and thus disrupt the relative balance in the microbiome in regard to structure and functionality. This could have serious consequences for human health. The risks that can emerge in these biological systems cannot be assessed simply by examining the individual components, it is essential that the whole assemblage is considered. These are known as holobionts.

(4) Cumulative risks

As explained, NGTs can be used to create new genotypes and traits in new and different ways, and with different results to those achieved with older genetic engineering methods or conventional breeding (including random mutagenesis). Many organisms generated with NGTs across many species and with different traits may soon be released into the environment. Cumulative adverse effects arising from such releases may be more or less likely, depending on their specific biological characteristics (intended or unintended).

Large-scale releases may increase the likelihood of such effects. Interactions between the species or between the various traits within an individual species may also occur. These interactions have the potential to cause adverse effects which may be direct or indirect, immediate, delayed or cumulative.

In general, effects emerging from interactions may be additive, antagonistic or synergistic and are hard to predict. Both the intended and unintended effects resulting from the NGT processes have to be taken into account. Even if each of the individual NGT-GMOs were thought to be 'safe', uncertainties or even unknowns will still emerge in the combination of the 'events'. Therefore, the risk assessment of single events remains necessary, but it may fail to predict or assess short-term or long-term cumulative effects, or possible interactions with the receiving environment, or the combination of several traits.

A new dimension of hazards

The report describes various traits created with NGTs that have the potential to cause harm to human health and the environment (see, for example, EFSA, 2010). In addition, the potential to cause harm will depend on the exposure and the ability of the organisms to spread and persist in the environment. Hazards include the disturbance or disruption of ecosystems as well adverse health effects at the stage of consumption.

NGTs will basically create a whole new dimension of hazards and threat potential: the introduction of tools, such as CRISPR/Cas, enables a new depth of technical intervention into the genome that, for example, can result in extreme variations within the traits and unintended genetic changes which are unlikely to occur in conventional breeding. These effects are being generated within a rapidly developing field with an increasing number of applications. Many applications are not confined to domesticated plants or animals. Instead, we are seeing an increasing number of projects looking at wild populations and a broad range of organisms such as microorganisms, insects, rodents and trees, all of which are embedded in their own complex ecosystems.

Similarly to environmental pollution with plastics and chemicals, it is not always an individual NGT-GMO which may create the real problems, but rather the sum of diverse effects on the environment. The environmental problems created by the release of NGT-GMOs may not only be more diverse and complex but also last longer than those caused by plastics and pesticides – thus impacting many future generations.

These kinds of novel hazards triggered by potential releases of NGT-GMOs can rapidly overwhelm the adaptability of ecosystems. NGT-GMOs may, in addition to other man-made crises such as climate change, represent a further destabilization in ecosystems or further intensify specific unfavorable effects. This is the reason why the release of genetically engineered organisms into the environment should be restricted.

Requirements for risk assessment and risk research

As shown, each NGT application has the potential to cause specific intended or unintended genetic alterations in the genome. Therefore, the risks and hazards associated with NGT-GMOs cannot simply be categorized into risk groups according to their intended traits. There is no scientific justification for the *a priori* exclusion of groups of NGT-GMOs from mandatory risk assessment on the basis of establishing 'risk profiles' as proposed by the EU Commission.

On the contrary, this has served to highlight the need for the European Food Safety Authority (EFSA) to carry out much more exact analysis. The EFSA has never actually been asked to present a detailed risk analysis in regard to NGTs in which the unintended effects are also systematically taken into account. The same is true for effects emerging from interactions between NGT-GMOs that may be direct or indirect, immediate, delayed or cumulative. Against this backdrop, the EU Commission should request EFSA to explore and answer to the following questions:

Which requirements are necessary and which methods are suitable for:

- detecting and assessing unintended genetic or epigenetic alterations caused by the processes of NGT ?
- detecting and assessing unintended effects of the intended NGT traits on the level of the organisms?
- detecting and assessing intended and unintended effects of the NGT-GMOs in ecosystems?
- detecting and assessing specific intended and unintended effects caused by the processes of NGT with relevance for food & feed safety?
- detecting and assessing cumulative effects and interactions between different NGT-GMOs?
- establishing precautionary measures to prevent uncontrolled spread in the environment?

Technology assessment (TA)

Some stakeholders and political decision-makers are seeking to create the impression that the hypothetical benefits of NGT-GMOs are a given fact. However, as yet there is no established regulatory system to provide sufficiently clear and transparent standards or criteria needed to make evidence-based decisions on sustainability and potential benefits. Unless there are sufficiently defined standards in place, misinformation and market distortion remain significant risks.

The introduction of transgenic plants thirty years ago came with high expectations in regard to the reduction of pesticides and beneficial health effects. However, many of these expectations failed to materialize. How can we avoid similar developments in the context of NGT-GMOs? Guidance and criteria may be needed to distinguish traits with 'real benefits' from those which are simply 'empty promises'. These criteria also are needed to achieve the goals of the EU as set out in the 'Green Deal' and its 'Farm to Fork' strategy. In worst case, insufficiently regulated NGTs may block systemic options needed to achieve more sustainability. Since these traits are often associated with 'trade-off' reactions (side effects) that can make it necessary to invest much more time into developing a trait compared to conventional breeding. In summary, NGTs, e.g. CRISPR/Cas, have huge potential to alter the genome but this potential does not easily translate into real benefits.

Therefore, in addition to a mandatory case-by-case risk assessment, the priority for political decision-makers should be a complementary regulatory framework for prospective TA. It should include robust criteria to assess the potential benefits of NGT-GMOs for production systems and the environment.

There are complex issues that need to be considered: it can be assumed that if NGT-GMOs are introduced into agriculture on a large-scale, this will not only affect the characteristics of distinct crops and livestock, but it will also have an extensive impact on food production systems as a whole. In general, NGTs should be considered to be disruptive technologies which will impact ecosystems as well as social and economic systems of food production if introduced into agriculture on a large-scale.

For example, NGTs may disrupt existing systems in regard to coexistence, labeling and traceability if the EU regulations are weakened or fragmented. Under these circumstances, freedom of choice for consumers, organic agriculture and non-GE food production may be severely hampered or disabled. Food security, food sovereignty and freedom of choice for farmers and consumers should, therefore, be taken into account in prospective TA.

Other important issues include the accessibility of the proprietary technology and access to biological material needed for all innovation in breeding. In particular, patents play a crucial role in this context as they can be used to block, hamper or control access to both technology and biological resources.

While TA cannot replace the risk assessment of the specific organisms (events), it is nevertheless necessary for making political decisions and in seeking a balance between potential benefits and the need to reduce the

overall risk of adverse effects on biodiversity and human health. However, in the context of NGTs, the methodology for a comprehensive TA still needs to be developed.

TA should also take into account alternatives which are based on conventional breeding, agroecology or other knowledge gained from food production systems. As shown in the report, there are many traits which are potentially beneficial for sustainable agriculture goals or climate change mitigation that can be derived from conventional breeding methods. Presented with these conditions, it appears obvious that traditional breeding methods, which are less risky and create less uncertainty, should be given priority. Furthermore, research in agroecological sciences shows that types of agricultural systems as a whole, will have much greater influence in regard to sustainability and mitigating the effects of climate change than the traits of individual varieties.

Requirements for NGT regulation against the backdrop of the precautionary principle

The overarching question to be answered in this report is: *“What are the crucial requirements for an ideal regulation of NGTs from the perspective of the consumers and the protection goals regarding health, the environment and animal welfare?”*

The following short summary presents some answers from the report:

EU regulation of NGTs should prevent:

- uncontrolled marketing or releases of NGT-GMOs organisms into the environment;
- damage to biological diversity, ecosystems and agriculture;
- health hazards being introduced unnoticed into the food system where they might accumulate;
- data needed for risk assessment by independent experts being treated as confidential business information;
- contamination of organic and other food or seed production systems that exclude the use of genetically engineered organisms.

EU regulation of NGTs should ensure:

- a case-by-case risk assessment and an approval process for each NGT event, including taking accumulated effects into account;
- the further development of data requirements, guidelines and methods of risk assessment to achieve the highest safety standards, including cut-off criteria in cases where uncertainty is too great;
- the availability of information to track and trace the NGT-GMOs and food products derived thereof;
- measures are in place to prevent the uncontrolled spread of NGT-GMOs in the environment;
- consumer choice and coexistence with organic and GE free food production;
- animal welfare is fully respected at all stages of the NGT processes;
- prospective and comprehensive TA is carried out before NGTs are brought to market.

Consequently, all NGT-GMOs need to undergo a mandatory approval process before being released into the environment or brought to market. Risk assessment should (as currently requested by EU legislation) aim to identify the intended and unintended changes resulting from the technical processes of genetic engineering and should evaluate their potential to cause adverse effects on human health and the environment. In this context, direct and indirect effects which may be immediate, delayed or cumulative have to be taken into account (see Figure 2).

Furthermore, a comprehensive and prospective TA is essential prior to use in an agricultural setting in order to address systemic risks to biodiversity, socio-economic impacts and effects in regard to sustainability. Any such TA should be in accordance with the precautionary principle and, at the same time, evaluate the actual need to apply the technology and also the consider alternatives that could be made available. The single overarching principle should be to generally restrict releases of NGT-GMOs into the environment to avoid, e.g. passing potential tipping points that would cause irreversible damage to ecosystems.

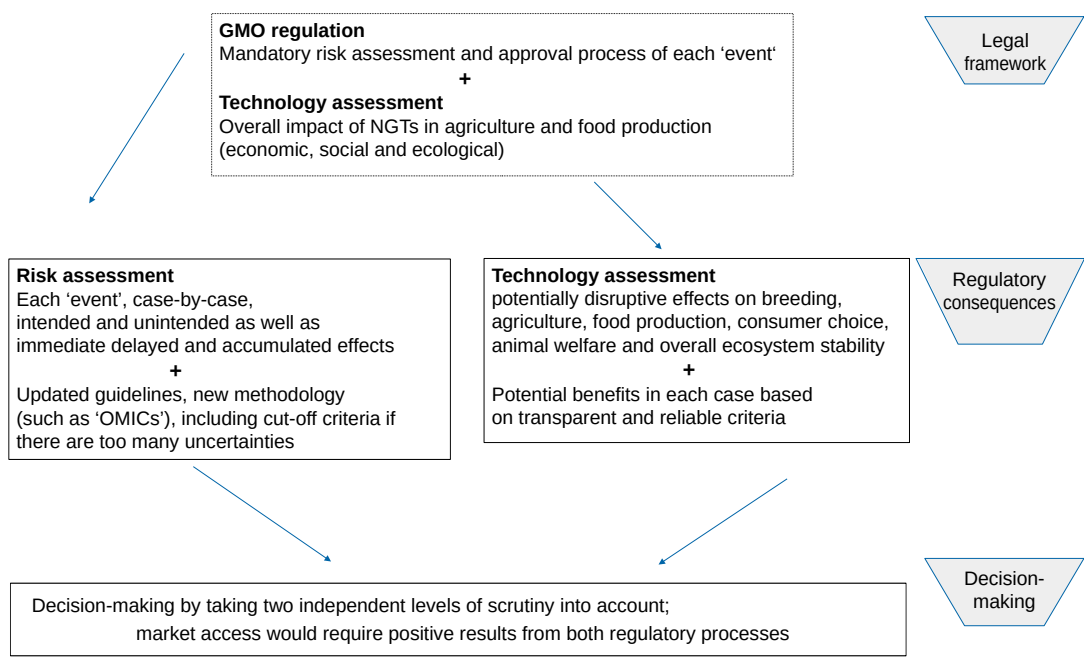


Figure 2: Improving the EU GMO regulatory framework from the perspective of the precautionary principle by updating risk assessment standards and introducing a corresponding framework for a technology assessment

Zusammenfassung

Dieses Gutachten dient dazu, Fragen des Verbraucherzentrale Bundesverbandes (vzbv) im Zusammenhang mit der EU-Regulierung neuer genomischer Techniken (NGT, auch Neue Gentechnik, Genome Editing) zu beantworten. Zu diesem Zweck gibt das Gutachten einen Überblick zu verschiedenen Aspekten die insbesondere im Bezug auf Landwirtschaft und Lebensmittelproduktion wichtig sind.

Hintergrund der Fragen sind Initiativen der EU-Kommission, die bestehende Gentechnik-Gesetzgebung zu ändern. In diesem Zusammenhang stellt der vzbv die (übergreifende) Frage: „*Welches sind die zentralen Anforderungen an eine aus Verbraucher-, Umwelt- und Tierschutzsicht optimale Regulierung neuer gentechnischer Verfahren?*“

Die zusätzlichen Fragen, die die Grundlage für das Gutachten sind, lauten:

- a. *Was spricht dagegen, bestimmte neue Gentechnikverfahren von der europäischen Gentechnikregulierung auszunehmen?*
- b. *Welche Erkenntnisse liegen bezüglich der Nachweisbarkeit neuer Gentechnikverfahren vor und was bedeutet dies für die Risikobewertung, Zulassung, Rückverfolgbarkeit und Kennzeichnung der neuen Gentechnik?*
- c. *Welche Erkenntnisse liegen über die nicht beabsichtigten Effekte vor und wie sollten sie in einer Risikobewertung berücksichtigt werden?*
- d. *Welche Erkenntnisse liegen darüber vor, dass neue gentechnische Verfahren spezifische Risiken aufweisen?*
- e. *Welche Gründe sprechen für die Notwendigkeit einer „Fall-zu-Fall“-Bewertung (Einzelfallrisikobewertung und -zulassung) und unter welchen Voraussetzungen (Nachweisführung, Belege der Unbedenklichkeit) könnte eine ganze Gruppe von Gentechnikverfahren einheitlich gehandhabt werden?*
- f. *Wie könnten Veränderungen nicht nur bezüglich der beabsichtigten Eigenschaften einer Pflanze untersucht und bewertet werden, sondern auch in Bezug auf die unbeabsichtigten?*
- g. *Welche Erkenntnisse liegen darüber vor, dass Veränderungen, die durch neue gentechnische Verfahren erzeugt werden, sich von natürlich entstandenen Mutationen unterscheiden?*
- h. *Wie belastbar ist das Argument, nur mit neuen Gentechnikverfahren könnten sich an das verändernde Klima angepasste Pflanzen erzeugt werden?*
- i. *Wie ist der Einsatz der neuen Gentechnik in der Tierzucht zu beurteilen?*

Um diese Fragen zu beantworten, gibt das Gutachten einen Überblick zu verschiedenen Aspekten, insbesondere in Zusammenhang mit Landwirtschaft und Lebensmittelproduktion. Das Gutachten erläutert technische Eigenschaften der NGTs und mögliche Anwendungen in der Landwirtschaft und Lebensmittelproduktion. Diese werden in mehreren Beispielen veranschaulicht, ebenso wie die Risiken mit Bezug auf Pflanzen, Tiere und Mikroorganismen. Schließlich wird ein Überblick über Anforderungen an die Regulierung und die Untersuchung der Risiken gegeben.

Was ist neu an der Neuen Gentechnik?

Mit der Hilfe der NGTs können genetische Veränderungen herbeigeführt werden, die über das hinausgehen, was mit der konventionellen Züchtung erreicht wird, ohne dafür zusätzliche Gene einfügen zu müssen. Anders als die konventionelle Züchtung (einschließlich der Zufallsmutagenese) können NGTs die Beschränkungen der natürlichen Genomorganisation überschreiten, wie sie von der Evolution hervorgebracht wurden. Insbesondere die ‚Gen-Schere‘ CRISPR/Cas macht das Erbgut, im Vergleich mit früheren Methoden der Züchtung, in größerem Umfang für Veränderungen verfügbar.

Die höhere Verfügbarkeit des Genoms macht es möglich, dass

- grundlegende Veränderungen der biologischen Eigenschaften von Organismen herbeigeführt werden können, auch wenn keine zusätzlichen Gene eingefügt werden;
- die Beschränkungen der natürlichen Genomorganisation, wie zum Beispiel Reparaturmechanismen (oder andere Schutzfaktoren wie Gen-Duplikationen) überwunden werden und so neue Genotypen generiert werden, die über das hinausgehen, was mit konventioneller Zucht erreicht wird;
- extremere Versionen bekannter Phänotypen oder auch neue Phänotypen erzielt werden, die oft mit Nebenwirkungen („trade offs“) verbunden sind.

Zudem gehen die technischen Potentiale von Werkzeugen wie CRISPR/Cas auch mit einem großen Potenzial für unbeabsichtigte genetische Veränderungen einher, die unter den Gegebenheiten der konventionellen Zucht kaum zu erwarten sind.

Dabei gibt es mehrere Faktoren, die die Ergebnisse der NGT-Verfahren in Bezug auf beabsichtigte und unbeabsichtigte Eigenschaften beeinflussen. Dazu gehören die jeweilige Art, die intendierten Züchtungsmerkmale, die Zielgene (ihre Position im Erbgut, Funktion, Anzahl, Ähnlichkeit mit anderen Genen), der Typ der ‚Gen-Schere‘ (oder anderer technischer Hilfsmittel) und der Prozess der Einschleusung der Gen-Schere in die Zellen (bzw. der von anderen technischen Hilfsmitteln).

Mögliche Anwendungen in Landwirtschaft und Lebensmittelerzeugung

Die Bandbreite der Arten, die mithilfe der NGTs verändert werden, geht weit über das hinaus, was mit den bisherigen („alten“) gentechnischen Verfahren erreicht wurde. Allerdings kann die Effektivität der Verfahren von Fall zu Fall unterschiedlich sein. Betroffen sind eine große Anzahl von Pflanzen und Tieren, die zur Erzeugung von Lebensmitteln genutzt werden, aber auch nicht-domestizierte Arten, zu denen Bäume, andere Pflanzen, Insekten, Wirbeltiere und Mikroorganismen gehören. Viele der Arten, bei denen die NGTs zur Anwendung kommen, haben auch das Potential, über längere Zeiträume in der Umwelt zu überdauern und sich auszubreiten.

Einige Beispiele für relevante NGT-Anwendungen wurden in verschiedenen Datenbanken recherchiert und umfassen Pflanzen, Tiere, Speisepilze und Mikroorganismen. Allerdings kann aus diesen Daten nicht abgeleitet werden, welche von diesen NGT Organismen tatsächlich den Markt erreichen werden.

Pflanzen und Speisepilze

Die angestrebten Züchtungsmerkmale bei Pflanzen umfassen u.a. Veränderungen in der Zusammensetzung der Inhaltsstoffe, Stresstoleranz (gegenüber biotischen und abiotischen Faktoren), Ertrag, Herbizidresistenz und verbesserte Haltbarkeit. Das Gutachten gibt einen Überblick über Pflanzen, die bereits vermarktet werden (Tomaten mit angeblich gesundheitsförderlichen Inhaltsstoffen und Sojabohnen mit veränderter Ölzusammensetzung). Andere Beispiele betreffen Speisepilze mit verzögerter Bräunung, herbizidresistenten Mais, Leindotter mit veränderter Ölzusammensetzung, neu domestizierte (Wild-) Tomaten und Weizen (mit verschiedenen Eigenschaften). Zu jedem Beispiel werden die beabsichtigten und die unbeabsichtigten Eigenschaften erörtert.

Tiere

Die NGT Anwendungen bei Tieren, die der Lebensmittelgewinnung dienen, umfassen die Fleischqualität und höhere Fleischausbeute ebenso wie die Anpassung an den Klimawandel und die Haltungsbedingungen. Das Gutachten gibt einen Überblick über die Tiere, die bereits vermarktet werden könnten (zwei Fischarten mit verändertem Wachstum in Japan und Rinder mit dünnerem Fell in den USA). Weitere Beispiele umfassen hornlose Rinder und Hennen, die keine männlichen Nachkommen produzieren. Zu jedem Beispiel werden die beabsichtigten und die unbeabsichtigten Eigenschaften erörtert.

Mikroorganismen und Viren

Anwendungen von NGTs können auch Mikroorganismen wie Bakterien, Archaeen, Pilze und Hefen umfassen, in einigen Fällen auch Viren. Die möglichen Ziele betreffen u.a. die Bodenqualität, das Mikrobiom von Pflanzen und Tieren, Pestizide und ‚Paratransgenese‘ (Beeinflussung der Eigenschaften von Pflanzen oder Tieren durch Veränderung ihrer assoziierten Mikrobiota).

Um welche Risiken geht es?

Sowohl die beabsichtigten als auch die unbeabsichtigten genetischen Veränderungen, die durch die NGTs verursacht werden, unterscheiden sich oft deutlich von denen, die aus konventioneller Züchtung (einschließlich Zufallsmutagenese) resultieren. Diese spezifischen Unterschiede zwischen NGTs und natürlichen Prozessen (bzw. konventionellen Verfahren zur Züchtung) sind in manchen Fällen leicht zu übersehen, können aber schwerwiegende Konsequenzen haben. Dabei müssen direkte und indirekte Effekte berücksichtigt werden, die unmittelbar, verzögert oder kumulativ sein können.

(1) Risiken, die mit den beabsichtigten Eigenschaften von Pflanzen und Tieren einhergehen

Wie im Falle von Leindotter (mit verändertem Ölgehalt), Weizen (zur Reduktion von Gluten in Backwaren) und Fischen (schneller an Gewicht zunehmende Meeresbrassen und Kugelfische) gezeigt wird, sind die Eigenschaften, die mit NGTs bewirkt werden, oft extreme Ausformungen biologischer Eigenschaften oder auch neue Eigenschaften, die aus konventioneller Züchtung so kaum zu erwarten sind. Diese Eingriffstiefe kann zu Nebenwirkungen (Ausgleichsreaktionen) im Stoffwechsel der Organismen führen, die beispielsweise die Stresstoleranz von Pflanzen schwächen oder auch Tierschutzprobleme hervorrufen können.

Diese direkten und indirekten Effekte sind das Ergebnis von Wechselwirkungen im komplexen Netzwerk der Gene, Proteine und anderer biologisch aktiver Moleküle. Derartige unbeabsichtigte Stoffwechselwirkungen und physiologische Reaktionen können auch dann eintreten, wenn der Eingriff ins Erbgut gezielt und präzise ist.

Zusammengefasst können die Effekte, die durch die beabsichtigten Eigenschaften verursacht werden, weitreichende Auswirkungen auf die Umwelt, die Gesundheit der Pflanzen und Tiere als auch auf den Ernteertrag, den Einsatz von Pestiziden und die Lebensmittelsicherheit haben. Werden die gentechnisch veränderten NGT-Organismen (NGT-GVOs) freigesetzt, können Wechselwirkungen mit der Umwelt, einschließlich Schädlingen, Pathogenen und Klimafaktoren, die Komplexität der jeweiligen Risikoszenarien wesentlich erhöhen.

(2) Risiken unbeabsichtigter gentechnischer Veränderungen von Pflanzen oder Tieren

Ähnlich wie im Falle der beabsichtigten Eigenschaften, können auch unbeabsichtigte genetische Veränderungen zu Genotypen führen, die sich von denen unterscheiden, die aus konventioneller Züchtung resultieren. Diese Effekte umfassen DNA-Brüche in Nichtzielregionen, repetitive Deletionen bestimmter DNA-Abschnitte, Insertionen und Deletionen (Indels) unterschiedlicher Größe, umfangreiche strukturelle Veränderungen in der Zielregion und der unbeabsichtigte Einbau von Transgenen. Werden diese unbeabsichtigten Effekte übersehen, können sie sich rasch in größeren Populationen ausbreiten. Als Beispiel sind hier hornlose NGT-Rinder zu nennen, bei denen erst nach einigen Jahren festgestellt wurde, dass auch Gene aus Bakterien, inklusive einer Resistenz gegen Antibiotika, ungewollt in das Erbgut der Tiere gelangt waren. Glücklicherweise waren diese Tiere noch nicht in der kommerziellen Zucht eingesetzt worden. Ansonsten hätten sich diese unerwünschten Merkmale rasch in den Zuchtbeständen ausbreiten können. Das gleiche Problem besteht, wenn Saatgut mit fehlerhaftem Erbgut über längere Zeit für die weitere Vermehrung und Züchtung eingesetzt wird. Unter Umständen können die Fehler im Erbgut auch akkumulieren.

In diesem Zusammenhang muss beachtet werden, dass die Verfahren der Neuen Gentechnik mehrere Stufen umfassen, die mit inhärenten Risiken einhergehen und unabhängig von den jeweiligen Zielen (beabsichtigten Eigenschaften) sind. Beispielsweise wird beim Einsatz der Gen-Schere CRISPR/Cas in Pflanzen regelmäßig auf die ungezielten Verfahren der alten Gentechnik zurückgegriffen, um die DNA, die für die Bildung der Gen-Schere notwendig ist, in die Zellen einzubringen. Daher sind das Ergebnis des Einsatzes von CRISPR/Cas bei Pflanzen (in den meisten Fällen) zunächst transgene GVOs, die eine Vielzahl von unbeabsichtigten genetischen Veränderungen aufweisen können. Erst am Ende des mehrstufigen Verfahrens werden mit Hilfe von üblichen züchterischen Verfahren die transgenen Abschnitte wieder aus dem Erbgut der Pflanzen entfernt (Segregationszüchtung). Fehlen ausreichende Standards für die Risikobewertung, können die unbeabsichtigten Effekte unbemerkt im Erbgut überdauern und sich rasch in den Populationen ausbreiten und ggf. auch akkumulieren.

Im Ergebnis können auf jeder Stufe des Prozesses – (i) der Insertionen der DNA für die Gen-Schere, (ii) der Erkennung und der Veränderung der Zielregion und (iii) den Reparaturprozessen in den Zellen – spezifische unbeabsichtigte Veränderungen auftreten, die mit Risiken einhergehen (siehe Abbildung 1).

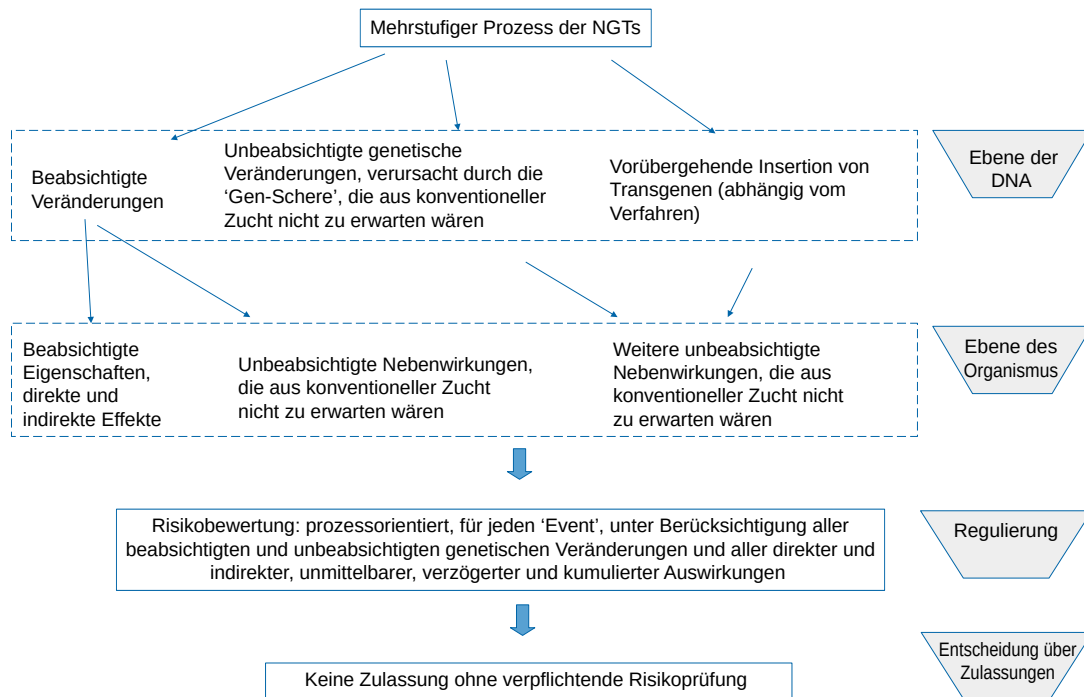


Abb. 1: Beabsichtigte biologische Merkmale und deren Nebenwirkungen sowie unbeabsichtigte genetische Veränderungen die für die Risikobewertung bei Tieren und Pflanzen wichtig sind und einen Zulassungsprozess notwendig machen.

(3) Risiken in Zusammenhang mit Mikroorganismen

Nach einer Freisetzung können gentechnisch veränderte Mikroorganismen in der Umwelt überleben und überdauern. Zudem können auch neue Lebensräume erobert und dabei vielfältige Wechselwirkungen mit anderen Organismen eingegangen werden. Auch wenn Mikroorganismen nur für den Einsatz in geschlossenen Systemen vorgesehen sind, können sie sich unter Umständen in der Umwelt ausbreiten: Erfahrungen im Umgang mit Mikroorganismen, die im Rahmen der Nahrungsmittelproduktion eingesetzt werden, zeigen, dass diese in großem Umfang zu Kontaminationen von Lebens- und Futtermitteln führen können. Darunter befinden sich sowohl lebensfähige gentechnisch veränderte Mikroorganismen als auch nur deren Erbgut.

Generell sind viele Mikroorganismen eng mit Arten aus anderen Domänen (Pflanzen oder Tiere) assoziiert, die auch als deren ‚Wirt‘ bezeichnet werden. Spezifische Zusammensetzungen von Mikroorganismen bilden das Mikrobiom von Pflanzen, Insekten, Wirbeltieren und auch von Menschen. Freisetzen von Mikroorganismen aus NGT Anwendungen können in diesen Mikrobiomen überdauern, sie kolonialisieren und auch in ihrer Balance (im Hinblick auf Struktur und Funktionalität) stören. Dies kann auch zu erheblichen gesundheitlichen Beeinträchtigungen von Verbraucher:innen führen. Die Risiken, die von solchen biologischen Systemen ausgehen, lassen sich nicht allein durch die Betrachtung isolierter einzelner Teile und Fragmente beurteilen, vielmehr müssen alle beteiligten Organismen und Interaktionen als größere Einheiten, so genannte Holobionten, betrachtet werden.

(4) Kumulative Risiken

Wie erläutert können mit Hilfe von NGTs neue Genotypen und biologische Eigenschaften auf eine andere Art und Weise und mit anderen Ergebnissen erzielt werden, als mit den bisherigen gentechnischen Verfahren oder den Methoden konventionellen Züchtung (einschließlich der Zufallsmutagenese). Schon bald könnten NGT-GVOs, die viele Arten umfassen und eine große Bandbreite unterschiedlicher Eigenschaften aufweisen, in die Umwelt entlassen werden. Kumulative Schäden, die aus diesen Freisetzungen resultieren, können in Abhängigkeit von den beabsichtigten oder unbeabsichtigten Eigenschaften mehr oder weniger wahrscheinlich sein, wobei Freisetzungen im großen Maßstab die Wahrscheinlichkeit für derartige Effekte erhöhen. Wechselwirkungen treten möglicherweise zwischen den Arten oder auch zwischen verschiedenen Eigenschaften innerhalb einer Art auf. Diese Wechselwirkungen können direkt oder indirekt, unmittelbar, verzögert oder auch kumulativ sein.

Im Allgemeinen sind Wechselwirkungen, die aus additiven, antagonistischen oder synergistischen Effekten hervorgehen, schwer vorherzusagen. Dabei sind sowohl die beabsichtigten als auch die unbeabsichtigten Effekte zu berücksichtigen. Selbst wenn einzelne NGT-GVOs als sicher angesehen werden, können sich aus der Kombination ihrer Eigenschaften neue Unsicherheiten und Risiken ergeben. Es ist also sowohl eine Risikobewertung aller einzelnen NGT-GVOs notwendig, als auch ihrer kumulativen Effekte und möglicher Wechselwirkungen mit der Umwelt oder anderen NGT-GVOs.

Eine neue Dimension der Gefährdung

Im Gutachten werden verschiedene Eigenschaften von GVOs vorgestellt, die aus NGT-Verfahren stammen und zu Gefahren für Mensch und Umwelt führen können. Das Gefährdungspotential wird zusätzlich von der Exposition mit diesen Organismen und ihrer Fähigkeit zur Persistenz und Ausbreitung in der Umwelt abhängen. Die möglichen Schäden betreffen die Störung der Ökosysteme als auch gesundheitliche Beeinträchtigungen beim Verzehr der Produkte.

Insgesamt implizieren die NGTs eine neue Dimension von Gefahrenpotentialen: Der Einsatz von Hilfsmitteln wie CRISPR/Cas ermöglicht eine neue Eingriffstiefe, die zu extremen Ausformung biologischer Eigenschaften oder auch unbeabsichtigten genetischen Veränderungen führen kann, die im Rahmen der konventionellen Züchtung nicht zu erwarten sind. Diese Effekte entstehen in einem dynamischen technischen Umfeld mit einer wachsenden Anzahl von Anwendungen. Viele davon sind nicht auf bereits domestizierte Pflanzen oder Tiere begrenzt, vielmehr gibt es eine wachsende Anzahl von Projekten, die auch Wildpopulationen und eine große Bandbreite von Organismen betreffen, die in komplexe Ökosysteme eingebettet sind, wie Mikroorganismen, Insekten, Nagetiere oder Bäume.

Ähnlich wie bei der Verschmutzung der Umwelt mit Plastik und Chemikalien muss es nicht immer ein bestimmter GVO sein, der die tatsächlichen Probleme verursacht, vielmehr kann die Gesamtheit unterschiedlicher Auswirkungen mehrerer GVOs auf die Umwelt entscheidend sein. Dabei können diese Umweltprobleme nicht nur wesentlich vielfältiger und komplexer sein, sondern auch länger in der Umwelt überdauern und somit viele zukünftige Generationen belasten.

Diese neue Dimension der Gefährdungspotentiale, die durch mögliche Freisetzungen von NGT-GVOs verursacht wird, kann die Anpassungsfähigkeit der Ökosysteme rasch überfordern. Dadurch können die NGTs zusätzlich zu den bereits existierenden menschengemachten Krisen wie dem Klimawandel zu einer weiteren Destabilisierung der Ökosysteme beitragen oder bestimmte nachteilige Effekte noch verstärken. Aus diesem Grund sollte die Einbringung von GVOs in die Umwelt in ihrer Gesamtheit möglichst begrenzt werden.

Anforderungen an die Risikobewertung

Wie im Gutachten dargelegt wird, gehen die Verfahren der NGTs in jedem Fall mit der Möglichkeit spezifischer Risiken einher, die aus beabsichtigten oder unbeabsichtigten Veränderungen des Erbguts resultieren. Daraus folgt, dass die daraus entstandenen GVOs nicht einfach aufgrund ihrer beabsichtigten Eigenschaften in bestimmte Risikogruppen eingeteilt werden können. Es gibt keine wissenschaftlich ausreichende Begründung dafür, Gruppen von NGT-GVOs auf der Grundlage von ‚Risikoprofilen‘ von einer gesetzlich verpflichtenden Zulassungsprüfung *a priori* auszunehmen, wie dies von der EU-Kommission vorgeschlagen wird.

Vielmehr gibt es in diesem Zusammenhang den Bedarf für eine wesentlich genauere Analyse durch die Europäische Lebensmittelbehörde (EFSA). Bisher hatte die EFSA nicht den Auftrag, eine umfassende Risikoanalyse von NGTs vorzulegen, bei der auch die unbeabsichtigten Effekte systematisch erfasst werden. Sie hat auch keinen derartigen Bericht veröffentlicht. Gleiches gilt laut EU-Gesetzgebung für Wechselwirkungen zwischen den Organismen, die direkt oder indirekt, unmittelbar, verzögert oder kumulativ sein können. Vor diesem Hintergrund sollte die EU-Kommission die EFSA damit beauftragen, folgende Fragen zu beantworten:

Welche Anforderungen sind notwendig und welche Methoden sind geeignet, um

- *unbeabsichtigte genetische Veränderungen, die durch die NGT-Verfahren verursacht werden, zu entdecken und zu bewerten?*
- *direkte und indirekte Effekte der beabsichtigten NGT-Eigenschaften auf der Ebene der Organismen zu entdecken und zu bewerten?*
- *beabsichtigte und unbeabsichtigte, direkte und indirekte Effekte der NGT-GVOs auf der Ebene der Ökosysteme zu entdecken und zu bewerten?*
- *beabsichtigte und unbeabsichtigte, direkte und indirekte Effekte zu entdecken und zu bewerten, die Einfluss auf die Sicherheit von Lebens- und Futtermitteln haben können?*
- *kumulative Effekte und Wechselwirkungen zwischen verschiedenen NGT-GVOs zu entdecken und zu bewerten?*
- *vorsorglich mögliche unkontrollierte Ausbreitungen in der Umwelt zu erkennen und zu verhindern?*

Technikfolgenabschätzung (TA)

Einige Akteur:innen und auch Politiker:innen erwecken den Eindruck, dass die hypothetischen Vorteile der NGT-GVOs eine bewiesene Tatsache wären. Doch bisher gibt es keine Regulierung, mit der ausreichend klare und transparente Standards festgelegt würden, um die Nachhaltigkeit oder andere mögliche Vorteile objektiv und verlässlich zu bewerten. Ohne ausreichend definierte Standards könnten sich Falschinformationen ausbreiten und Marktverzerrungen verursacht werden.

Schon die Einführung von transgenen Pflanzen vor rund 30 Jahren ging mit hohen Erwartungen bezüglich der Einsparung von Pestiziden und positiven gesundheitlichen Effekten einher. Doch viele dieser Erwartungen haben sich nicht erfüllt. Wie können ähnliche Entwicklungen in Zusammenhang mit NGTs vermieden werden? Offensichtlich bedarf es Leitlinien und Kriterien, um zwischen Eigenschaften unterscheiden zu können, die mit tatsächlichen Vorteilen einhergehen, und jenen, die nur ‚leere Versprechungen‘ sind. Diese Kriterien sind auch für die Erreichung der Ziele wichtig, die sich die EU in Zusammenhang mit dem ‚Green Deal‘ und der ‚Farm-to-Fork‘-Strategie gesetzt hat. Unzureichend regulierte NGTs könnten schlimmstenfalls notwendige systemische Optionen für mehr Nachhaltigkeit verbauen. Da die mit Hilfe der NGTs erzeugten biologischen

Eigenschaften oft Nebenwirkungen (Ausgleichsreaktionen) im Stoffwechsel der Organismen auslösen, kann die Erreichung von züchterischen Zielen viel mehr Zeit in Anspruch nehmen, als dies bei konventioneller Züchtung der Fall ist. Zwar haben Verfahren der NGTs ein großes Potential für genetische Veränderungen, aber es ist nicht einfach, dieses Potential in tatsächliche Vorteile umzusetzen.

Zusätzlich zu einer verpflichtenden ‚Case-by-case‘-Risikoprüfung sollte die Politik deswegen einen Schwerpunkt auf die Schaffung eines zusätzlichen Regelwerks legen, das Grundlage für eine vorausschauende Technikfolgenabschätzung sein kann. Hierfür müssten verlässliche Kriterien entwickelt werden, um mögliche positive Effekte der NGTs für die Produktionssysteme und die Umwelt zu bewerten.

Dabei müssen unterschiedliche Aspekte einbezogen werden: Es ist anzunehmen, dass, falls NGTs in der Landwirtschaft in großem Umfang eingesetzt werden, dies nicht nur Auswirkungen auf die Eigenschaften bestimmter Pflanzen oder Tiere haben wird, sondern auch auf die Art und Weise der Lebensmittelerzeugung insgesamt. Ganz allgemein sollten die NGTs als eine disruptive Technologie angesehen werden, deren Auswirkungen sowohl die Ökosysteme als auch die sozio-ökonomischen Zusammenhänge in der Lebensmittelerzeugung betreffen wird, falls sie in großem Maßstab eingeführt wird.

So können die NGTs die bestehenden Systeme für Koexistenz, Kennzeichnung und Rückverfolgbarkeit gefährden, wenn die bestehende Regulierung abgeschwächt oder fragmentiert werden würde. Unter diesen Umständen würde die Auswahl für Verbraucher:innen, die biologische Landwirtschaft und die gentechnikfreie Lebensmittelproduktion ernsthaft behindert oder sogar unmöglich gemacht. Daher sollte die Technikfolgenabschätzung sowohl die Ernährungssicherheit, als auch die Ernährungssouveränität, die Wahlfreiheit für die Landwirtschaft und für die Verbraucher:innen berücksichtigen.

Auch der Zugang zu den Technologien und zu biologischem Material, das von allen Züchter:innen für ihre Innovationen benötigt wird, ist im Hinblick auf die Technikfolgenabschätzung ausschlaggebend. Insbesondere Patente spielen hier eine entscheidende Rolle, da diese dazu dienen können, sowohl den Zugang zur Technologie als auch zur biologischen Vielfalt zu kontrollieren, zu behindern oder zu blockieren.

Zwar kann die Technikfolgenabschätzung nicht die Risikobewertung der NGT-GVOs ersetzen. Dennoch ist sie unverzichtbar für politische Entscheidungen, die eine Balance im Hinblick auf mögliche Vorteile der NGTs und die Notwendigkeit der Vermeidung von möglichen Schäden anstreben. Allerdings müssen die geeigneten Methoden im Zusammenhang mit den NGTs erst noch entwickelt werden.

Die Technikfolgenabschätzung sollte auch Alternativen in Betracht ziehen, die auf der konventionellen Züchtung, auf Erkenntnissen der Agrarökologie oder anderen Erfahrungen aus der Lebensmittelerzeugung beruhen. Wie im Gutachten gezeigt, gibt es viele Eigenschaften, die mit den Mitteln der konventionellen Zucht erreicht werden können und positive Effekte im Hinblick auf die Ziele einer nachhaltigen Landwirtschaft und einer Abfederung des Klimawandels haben können. Unter diesen Umständen sollte im Zweifel den traditionellen Methoden der Züchtung, die mit weniger Risiken und Unsicherheiten einhergehen, Priorität gegeben werden. Zudem zeigen wissenschaftliche Erkenntnisse aus dem Bereich der Agrarökologie, dass die Art der Landwirtschaftssysteme in ihrer Gesamtheit einen wesentlichen größeren Einfluss im Hinblick auf Nachhaltigkeit und die Abfederung der Folgen des Klimawandel haben kann als die Züchtungsmerkmale einzelner Sorten.

Anforderungen an die Regulierung von NGTs im Lichte des Vorsorgeprinzips

Die übergeordnete Frage, die mit diesem Gutachten beantwortet werden soll, lautet: „*Welches sind die zentralen Anforderungen an eine aus Verbraucher-, Umwelt- und Tierschutzsicht optimale Regulierung neuer gentechnischer Verfahren?*“

Kurz zusammengefasst können aus diesem Gutachten folgende Antworten abgeleitet werden:

Die EU-Regulierung von NGTs muss verhindern, dass

- NGT-GVOs auf unkontrollierte Art und Weise freigesetzt oder vermarktet werden;
- Schäden an der biologischen Vielfalt, den Ökosystemen und der Landwirtschaft eintreten;
- sich gesundheitliche Gefahren unbemerkt in die Lebensmittelerzeugung einschleichen und akkumulieren;
- Daten, die für die Risikobewertung benötigt werden, als Geschäftsgeheimnis klassifiziert werden;
- ökologische und andere gentechnikfreie Produktionssysteme für gentechnikfreie Lebensmittel, Saatgut und Landwirtschaft kontaminiert werden.

Zudem muss die EU-Regulierung sicherstellen, dass

- jeder NGT-GVO einen Zulassungsprozess und die Risikoprüfung durchläuft und auch akkumulierte Effekte berücksichtigt werden;
- die Anforderungen an relevante Daten, Richtlinien und Methoden der Risikobewertung weiterentwickelt werden, um höchste Sicherheitsstandards zu gewährleisten; dazu gehören auch Ausschlusskriterien falls zu viele Unsicherheiten bestehen;
- die notwendigen Informationen vorhanden sind, um die NGT-GVOs und aus ihnen gewonnene Lebensmittel zu detektieren und zu verfolgen;
- Maßnahmen gegen eine unkontrollierte Ausbreitung von NGT-GVOs in der Umwelt ergriffen werden;
- die Wahlfreiheit für die Verbraucher:innen und die Koexistenz mit ökologischer und gentechnikfreier Lebensmittelproduktion gewährleistet ist;
- der Tierschutz auf allen Stufen der NGT-Verfahren vollumfänglich respektiert wird;
- eine umfassende und vorausschauende Technikfolgenabschätzung durchgeführt wird, bevor NGTs zugelassen werden.

Daraus folgt, dass alle NGT-GVOs einer verpflichtenden Zulassungsprüfung unterzogen werden müssen, bevor sie freigesetzt und/oder vermarktet werden können. Die Risikobewertung muss (wie derzeit von der EU-Gesetzgebung verlangt) alle beabsichtigten und unbeabsichtigten genetischen Veränderungen identifizieren, die durch den Prozess der gentechnischen Verfahren verursacht werden und diese im Hinblick auf mögliche Schäden für Mensch und Umwelt bewerten. Dabei müssen direkte und indirekte Effekte berücksichtigt werden, die unmittelbar, verzögert oder kumulativ sein können (siehe Abb. 2).

Zudem muss vor dem Einsatz von NGTs in der Landwirtschaft eine umfassende und vorausschauende Technikfolgenabschätzung durchgeführt werden, um sozio-ökonomische Auswirkungen, Effekte auf die Nachhaltigkeit und mögliche Kippunkte für die Ökosysteme zu bewerten. In Übereinstimmung mit dem Vorsorgeprinzip sollten dabei auch der tatsächliche Bedarf für NGTs sowie mögliche Alternativen im Detail geprüft werden. Dabei sollte es ein Ziel sein, die Freisetzungen der NGT-GVOs möglichst zu begrenzen, um bspw. mögliche Kippunkte, die zu irreversiblen Schäden an den Ökosystemen führen, zu vermeiden.

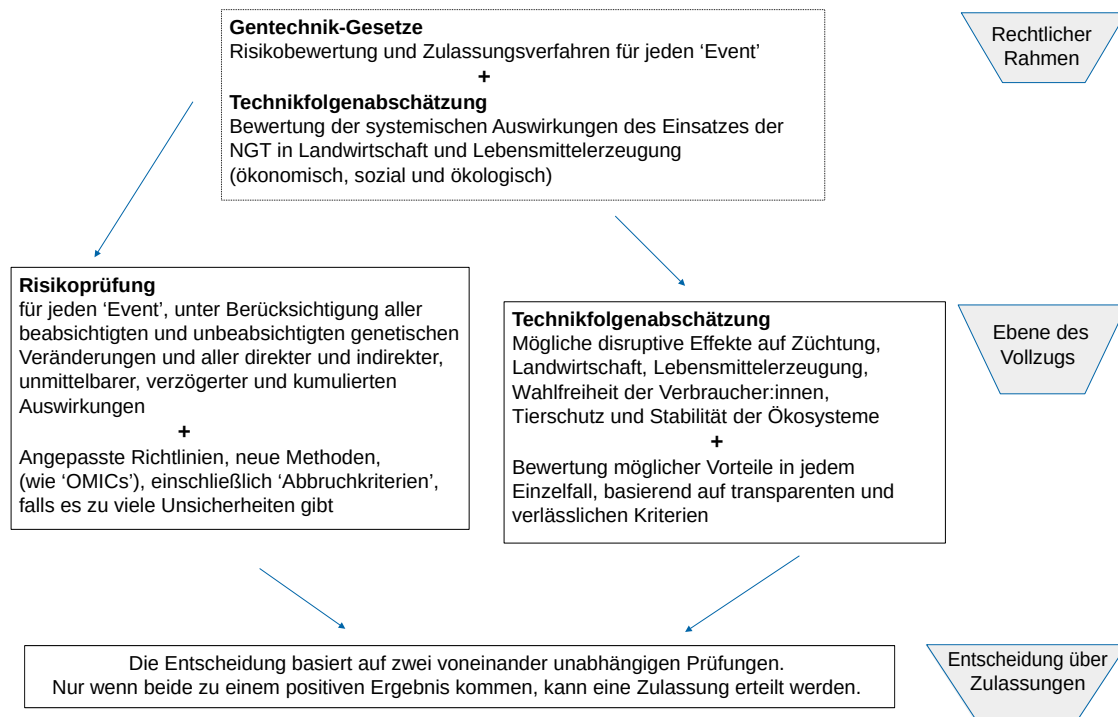


Abbildung 2: Ein mögliches Szenario für eine verbesserte Gentechnikgesetzgebung der EU aus der Perspektive des Vorsorgeprinzips: aktuelle Anpassungen der Richtlinien für die Risikobewertung und ein zusätzliches Regelwerk für die Technikfolgenabschätzung.

1. Introduction

This report was compiled to answer questions raised by the Federation of German Consumer Organisations (Verbraucherzentrale Bundesverband; vzbv) in the context of the EU regulation of new genomic techniques (NGTs, also new genetic engineering or genome editing). For this purpose, the report aims to provide an overview of several aspects which are especially relevant in the context of agriculture and food production.

The questions were raised because of EU Commission initiatives to change current EU GMO regulation. In this context, the (overarching) question raised by vzbv is “*What are the crucial requirements for an ideal regulation of NGTs from the perspective of the protection goals regarding health, the environment and animal welfare?*”

In addition, under the terms of reference (TOR) as provided by vzbv, the following questions were raised:

- a. *Are there any reasons not to exempt some specific groups of NGTs from EU GMO regulation?*
- b. *What is the current status of knowledge regarding identification of NGTs and what are the implications for risk assessment, the approval process, traceability and labeling of NGTs?*
- c. *What is the status of knowledge regarding unintended effects and how should they be taken into account in risk assessment?*
- d. *What is the status of knowledge regarding specific risks of NGTs?*
- e. *What are the reasons for a case-by-case risk assessment (approval process for each ‘event’) and under which conditions (burden of proof, evidence of safety) a whole group of NGTs could be handled in the same way?*
- f. *Which methods are available to not only assess the intended traits of a plant but also its unintended biological characteristics?*
- g. *What is the status of knowledge regarding the differences between genetic changes caused by NGTs and natural mutations?*
- h. *Are claims justified that NGTs are absolutely necessary to generate plants that are adapted to climate change?*
- i. *What are the pros and cons of NGT processes in animal breeding?*

Testbiotech was commissioned to deliver a report to answer these questions. By elaborating on these questions, Testbiotech also provides information on the scientific and legal background. Testbiotech has thus far made a substantial contribution to the scientific debate on the risks of NGT-GMOs from the perspective of the precautionary principle. In addition to several reports and backgrounders summarizing the current levels of scientific knowledge (Testbiotech, 2019a/b; Testbiotech, 2020a-d; Testbiotech, 2021a-e; Testbiotech, 2022; Testbiotech & CBAN, 2022), Testbiotech has been involved in several peer-reviewed publications on risks of NGTs (as authors or project holders) which were received with great interest by the scientific community (Kawall, 2019; Kawall, 2021 a/b; Kawall et al., 2020; Then et al., 2020). In addition, the author approached databases and other publications as referenced. If specific examples were chosen, the reasoning is explained. The author is, in particular, aware of the recent publications of EFSA as well as several consultations and reports published by the EU Commission on the future regulation of NGT-GMOs.

The organisms currently derived from NGTs are considered to be genetically engineered organisms that are regulated (as defined by EU Directive 2001/18/EC and Commission Directive (EU) 2018/350), and as such are subject to a mandatory approval process (as decided by the EU Court of Justice, Case C-528/16) before they can be released into the environment or introduced into the markets. Currently, all organisms derived from processes of genetic engineering are considered to be ‘events’, each characterized by the techniques applied, the steps in the process and the resulting intended and unintended effects. Commission Directive (EU) 2018/350,

requests that mandatory risk assessment must be applied for each ‘event’ and “shall identify the intended and unintended changes resulting from the genetic modification and shall evaluate their potential to cause adverse effects on human health and on the environment.” As further requested by EU regulation 2001/18/EC, direct and indirect effects which may be immediate, delayed or cumulative. The principles for risk assessment are laid down in Annex II of Directive 2001/18/EC which was amended by Commission Directive (EU) 2018/350. In addition, food safety has to be demonstrated in accordance with Regulation 1829/2003 and Commission Implementing Regulation 503/2013.

However, the EU Commission in recent reports (EU Commission 2021) and consultations¹ seems to be showing an intention to change current regulation. While no draft regulation was published, there appears to be an intention to harmonize EU standards and align them with standards in Canada as foreseen in the CETA bilateral trade agreement (Testbiotech & CBAN, 2022). Therefore, the introduction of ‘risk profiles’ might be based on the intended characteristics of the NGT-GMO and the unintended effects could be set aside.

These risk profiles might possibly be used to exempt specific categories of NGT-GMOs from the approval process or to require only more superficial safety checks. This political process may result in a fragmentation of the current GMO regulation, with only some ‘NGT events’ undergoing a mandatory approval process, but no longer foreseeing this requirement for all ‘events’ (see scenario in Figure 10).

As a result, it would cease to be the process used for the production of genetically engineered organisms that would trigger the mandatory risk assessment. At the same time, this means that all the intended and unintended changes that can have either direct or indirect, and either immediate or delayed effects on human health and on the environment, may no longer be subject to risk assessment as currently requested by Directive 2001/18/EC and Commission Directive (EU) 2018/350. Especially indirect, unintended, delayed and cumulative effects might be overlooked if these plans are put into regulatory practice. This political process is also one of the reasons for the wording of the question in the TOR.

This report, therefore, firstly gives an overview of the technical characteristics of NGTs with specific relevance to the questions in the TOR, followed by an overview of potential applications in the context of agriculture and food production. These examples include plants, animals, mushrooms and microorganisms. Organisms which are already being marketed (outside the EU) are also specifically considered. This information was then used to elucidate the risks for health and the environment and explore the requirements of future EU regulation. Further considerations include potential benefits and effects on the food production systems, including issues of consumer choice.

Since this report may also be used to inform the EU Commission or EU Member States, the term new genomic techniques (NGT), as established by the EU Commission, is used to summarize techniques which are also known as genome editing or new genetic engineering (New GE). Furthermore, the abbreviation GMO (genetically engineered organism) is used to mean organisms which are derived from genetic engineering (GE) as regulated by Directive 2001/18/EC.

1 https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/13119-Legislation-for-plants-produced-by-certain-new-genomic-techniques/public-consultation_en

2. Technical characteristics of NGTs

The following section of the report provides an overview of the technical potentials and technical characteristics of NGTs most relevant to the questions set out in the TOR in regard to an optimized regulation of NGTs from the perspective of the protection goals such as health, environment and animal welfare.

In this context, the ‘gene scissor’ CRISPR/Cas (Clustered regularly interspaced palindromic repeats/CRISPR associated) can be identified as the most important technical tool (see, for example, JRC 2021). It is, therefore, the main focus of the report.

2.1 Overview of some technical characteristics

NGTs allow new genotypes and traits to be generated in different ways and with different outcomes compared to previous genetic engineering methods or conventional breeding, including random mutagenesis (Eckersdorfer et al., 2019; Kawall, 2019; Kawall, 2021b). So-called site-directed nucleases (SDN), as used in CRISPR/Cas ‘gene scissors’ (Jinek et al., 2012; Doudna & Charpentier, 2014; EFSA, 2020a), are highly relevant in this context: they can be designed to target specific sites in the genome to knock out gene functions (SDN-1), to induce repair mechanisms for specific alterations of particular nucleotides to change specific gene functions (SDN-2), or to insert additional genes (SDN-3).

The nuclease CRISPR/Cas is currently the most relevant NGT-tool in the development of new plants (JRC, 2021). The nuclease is combined with an RNA that serves as a guide molecule and is designed to be specific for the DNA target site in the genome. After matching the guide RNA with the target region, the nuclease (which is strictly speaking the enzyme Cas) is then activated and typically cuts both strands of DNA (see also Figure 1). As a result, gene-functions will be disabled or changed.

Other relevant nucleases are TALENs (transcription activator-like effector nucleases) that were already established prior to the introduction of the CRISPR/Cas tool and are still applied in some cases. In addition, some variations on the Cas nuclease have been introduced recently (such as Cpf). There are nucleases which meanwhile appear to be of major importance (see JRC, 2021). All these nucleases can be categorized by using the SDN terminology in this report.

If the repair mechanisms are left to the process in the cells, this is called ‘non-homologous end joining’ (NHEJ). In these cases, no specific change in gene function is introduced, the intention is to simply knock out the natural gene functions (SDN-1). Typically, if the cell tries to restore the original gene function, the nuclease CRISPR/Cas can continue to cut until the intended incorrect repair is achieved and no more target sequence is available (Brinkman et al., 2018).

CRISPR/Cas might also be used to achieve specific changes to the gene functions (SDN-2 or SDN-3) via homologous recombination mediated by homology directed repair (HDR). In this case, additional DNA molecules are introduced alongside the Cas nuclease that serve as specific templates for the repair mechanisms which are meant to cause specific genetic alterations. The induced changes at or around the target site can be substitutions, deletions or insertions of one or more base pairs (SDN-2). If additional gene-sequences are inserted, the nucleases are classified as (SDN-3) (Eckersdorfer et al., 2019; Sander & Joung, 2014).

Depending on the specific SDN-1 or SDN-2 application, more extensive overall changes are possible. For example, multiplexing can target several genes simultaneously in a single application (Raitskin and Patron, 2016; Wang et al., 2016; Zetsche et al., 2017). Repeated applications of SDN-1 or SDN-2 can also be combined (Kawall et al., 2020). Changes involving the insertion of whole genes (including gene-stacking) are also possible (SDN-3) and are mediated by the use of specific donor DNA (Eckersdorfer et al., 2019; Sander & Joung, 2014).

If the outcome results in a genetically engineered organism inheriting a gene from another species, it is called 'transgenic'. If the outcome results in an organism with additionally inserted genes from the same species, it is called 'cisgenic'.

Further refinements, such as cutting only one strand of the DNA (nickase), the change of base pairs without cutting the strand of DNA (base editing) or specific variations that are meant to increase the efficiency and precision of the nucleases, may be applied. However, in regard to most of the plants (or animals) currently under consideration for being brought to market in near future, the SDN-1 processes as described above, are the ones that are applied in most cases (see JRC, 2021).

It should also be mentioned that there are some reports (such as EFSA, 2022a; EFSA 2022e) which assume the term cisgenesis (genetic engineering without introduction of genes from other species) can be used as a synonym for SDN-1 and also many SDN-2 or SDN-3 applications. In other EFSA reports (such as EFSA, 2021), the term SynBio is also used as a synonym for SDN applications.

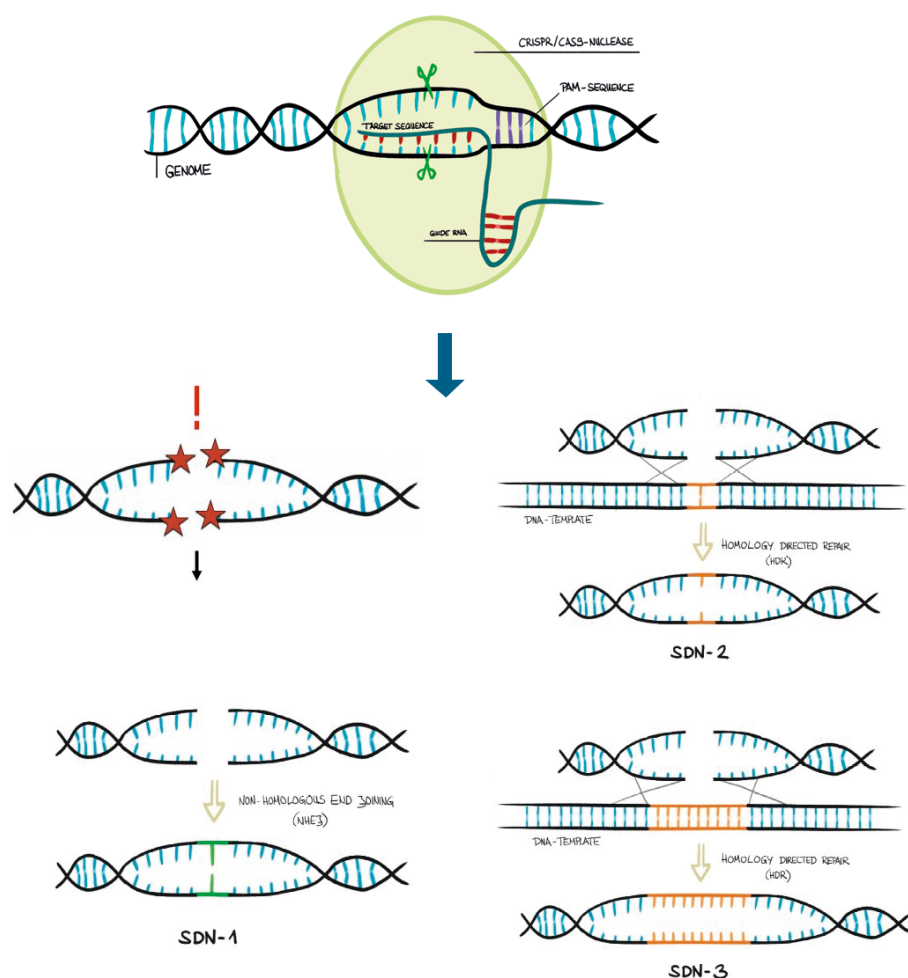


Figure 3: CRISPR/Cas (Clustered regularly interspaced palindromic repeats/CRISPR associated) can cause double stranded breaks of DNA at target sites in the genome and disable the repair mechanisms which may otherwise restore the original gene functions. It can knock out natural genes (SDN-1), introduce new gene functions (SDN-2) or insert additional genes (SDN-3).

2.2 What is 'new' about NGTs?

The following section provides an overview of some specific characteristics with general relevance for NGTs, in particular the CRISPR/Cas nuclease, in order to illustrate their technical potential:

a) Greater precision but a complex multistep process

NGTs can be used to introduce genetic changes with greater precision compared to previous techniques of genetic engineering. Typically, SDNs can be used to directly target the desired sites (Doudna & Charpentier, 2014; EFSA, 2020a, Jinek et al., 2012), whereas previous transformation processes introduce additional DNA sequences only at random sites (see, for example, Forsbach et al., 2003; Gelvin, 2017; Makarevitch et al., 2003). However, NGTs are based on processes involving several technical steps that, in case of plants, very often also include the older non-targeted transformation processes (such as biolistic methods² or the use of *Agrobacterium tumefaciens*³). These non-targeted methods are used to introduce the nucleases into the cells (Kawall et al., 2020) which may lead to unintended effects in many off-target regions (for example, see Yue et al., 2022). As pointed out in some publications, there are additional reasons why higher precision still seems to be challenging in several applications (Eckerstorfer et al., 2019; Kawall et al., 2020). In this context, there are several factors which impact the results of NGT processes in regard to the intended and unintended effects, such as the species, the trait, the target genes (their site, their function, their number, their similarities with other genes), the gene scissors (or other tools used) and the process of introducing the gene scissors (or other NGT tools) into the cells (see, for example, Kawall et al., 2020).

b) Overcoming the limitations of natural genome organization

NGTs can be used to achieve genomic changes extending beyond what is known from conventional breeding even without the insertion of additional genes. Compared to methods of conventional breeding (including random mutagenesis), NGTs can overcome the boundaries of natural genome organization that have evolved naturally from evolutionary processes. Relevant factors include repair mechanisms, gene duplications, genetic linkages and further epigenetic mechanisms (see, e.g. Belfield et al., 2018; Filler Hayout et al., 2017; Frigola et al., 2017; Halstead et al., 2020; Huang & Li, 2018; Jones et al., 2017; Lin et al., 2014; Monroe et al., 2022; Wendel et al., 2016), thus making the genome much more extensively available for genetic change (Kawall, 2019; Kawall et al., 2020). The resulting genotypes (the patterns of genetic changes) can be vastly different compared to those derived from conventional breeding, both in regard to intended and unintended changes (Kawall, 2021a/b), although there may still be some limitations to the effectiveness of the nucleases (Weiss et al., 2022). This means that it is possible to generate genotypes that are highly unlikely to result from natural processes or traditional breeding techniques, as well as create new phenotypes, including extreme versions of already known traits.

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- 2 Biolistic transformation is also known as particle bombardment or gene gun. It is a non-targeted method of genetic transformation of plants to deliver DNA into cells/tissues. The DNA to be introduced is coated onto small micro-particles which are 'shot' into the tissue at high pressure.
 - 3 *Agrobacterium tumefaciens* is a soil bacterium capable of parasitic growth on plants. The *agrobacteria* induce a genetic transformation in the host cell via stable integration of a DNA fragment called T-DNA. This mechanism of DNA transfer is a non-targeted method of genetic engineering using genetically engineered agrobacterium.

c) Changes in the allelic diversity within populations

Barbour et al. (2022) showed that a higher allelic diversity in plants has an impact on different species within an experimental food web, and may play a crucial role in the stability of ecosystems and food webs. CRISPR/Cas applications can, in particular, be used to make gene variants within a population more uniform, i.e. the frequency of the abundance of different allelic variants can be reduced, the alleles can be changed or the respective gene (-family) can be blocked in its functions. In this regard, CRISPR/Cas applications are very much more efficient than conventional breeding methods. Therefore, if NGT-GMOs are released into the environment, their impact on genetic diversity and associated ecosystems can extend far beyond what might be expected compared to natural processes and conventional breeding techniques.

d) Pervasive changes even without the insertion of additional genes

Even without the insertion of additional genes, changes in genotypes and phenotypes can be pervasive and brought about by, for example, knocking out very many or all copies of a gene family, thus changing several genes in parallel (multiplexing) or altering elements responsible for gene regulation (Kawall et al., 2020; Raitskin and Patron, 2016; Wang et al., 2016; Zetsche et al., 2017). Such technical interventions can lead to major and unprecedented changes in plant composition, which may also be associated with unintended effects (EFSA, 2022b; Kawall, 2021a/b; Nonaka et al., 2017; Sanchez-Leon et al., 2018).

e) Wide range of species and applications

The range of species that are accessible for NGTs extends far beyond applications of previously used techniques of genetic engineering. While effectiveness may differ from case to case, it does include a wide range of food plants and livestock, and also non-domesticated species comprising trees and other plants, insects, vertebrates and microorganisms, thus involving all domains of life (overview in: CBD, 2022; JRC, 2021; Testbiotech, 2021b). There are several specific applications designed for use in wild populations, including gene drives (Frieß et al., 2019; Gantz & Bier, 2015) and the intended release of genetically engineered viruses, also including Horizontal Environmental Genetic Alteration Agents (HEGAA) (Lentzos et al., 2022; Pfeifer et al., 2022). Many of the species targeted in NGT-applications also have the potential to persist and spread over longer periods of time without effective control. This may give rise to next generation effects not observed in the laboratory (Then et al., 2020).

f) Complex interactions also triggered by parallel releases

Large numbers of GMOs derived from NGTs, including various species with a wide range of different characteristics (intended or unintended), could be released into the same receiving environment within a short period of time (see, for example, JRC 2021). Depending on the scale of the release, its duration and the characteristics of the organisms, these NGT-GMOs may also intentionally or unintentionally interact with each other as well as with the 'original' receiving environment. In this context, a number of NGT-GMOs are designed for complex interactions, such as changes in the microbiome in the soil (Shelake et al., 2019; Shulze et al., 2019; Temme et al., 2012), in plants (Arif et al., 2020; Checcucci et al., 2018; Hettiarachchige et al., 2019; Vorholt et al., 2017), in insects (Bilgo et al., 2017; De Vooght et al., 2014; Fang et al., 2011; Gilbert et al., 2016; Leonard et al., 2018; Lovett et al., 2019; Leonard et al., 2020; Rangberg et al., 2012; Ren et al., 2008) or in corals (Levin et al., 2017). Moreover, some of the applications use a technique known as 'paratransgenesis' which aims to alter the biological characteristics of the host by genetically engineering its microbiome (Wilke et al., 2015).

2.3. Conclusions regarding differences and similarities of NGTs compared to previous methods and techniques

2.3.1 The most relevant differences compared to previous breeding

As mentioned firstly under 2.2.a), genome editing makes the genome of many species available for genetic changes to a greater extent compared to previous methods (Kawall, 2019). The CRISPR/Cas techniques can override the natural mechanisms in genome organization that protect essential genes (Belfield et al., 2018; Frigola et al., 2017; Halstead et al., 2020; Kawall, 2019; Monroe et al., 2022). Typically, the resulting changes would, unlike random mutations, not only alter a single copy of a non-target gene, but several or all copies (depending on plant species and degree of ploidy) (Kawall, 2021b). As a result, novel genotypes and biological characteristics can emerge from applications of this technology. The resulting organisms do not have a history of safe use (see Figure 4), and therefore their safety must be assessed before any release into the environment can be approved. These observations are relevant to both intended and unintended effects and their effects in the organisms as well as the interactions of the organisms with their environment. It may also concern food safety aspects.

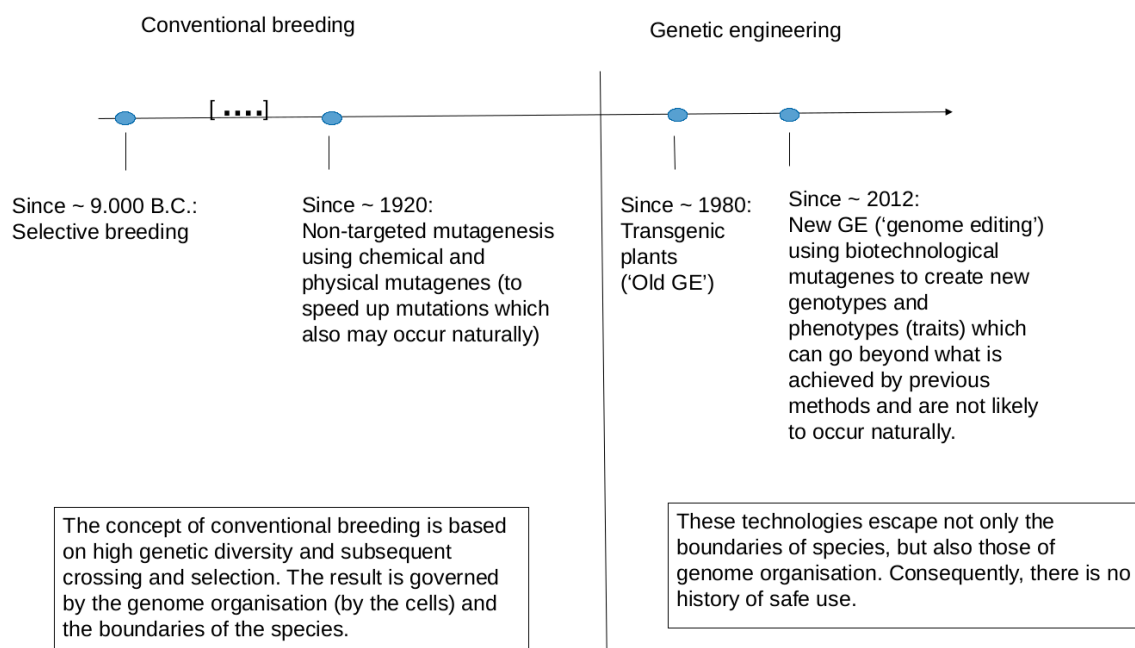


Figure 4: A historical perspective of the differences between plant breeding and genetic engineering.

2.3.2 The most relevant similarities compared to previous genetic engineering techniques

NGTs based on CRISPR/Cas applications are multi-step processes which, at least in plants, typically involve older genetic engineering ('Old GE'), such as non-targeted methods using *Agrobacterium* transformation or biolistic methods ('gene gun') to deliver the DNA for the nuclease ('gene scissors') into the cells (see Kawall et al., 2020 and Figure 5). Thus, in most cases, the result of the first step of the CRISPR/Cas application is a transgenic plant. Only at the end of the multistep process is conventional breeding used to remove the transgenic elements from the plant genome.

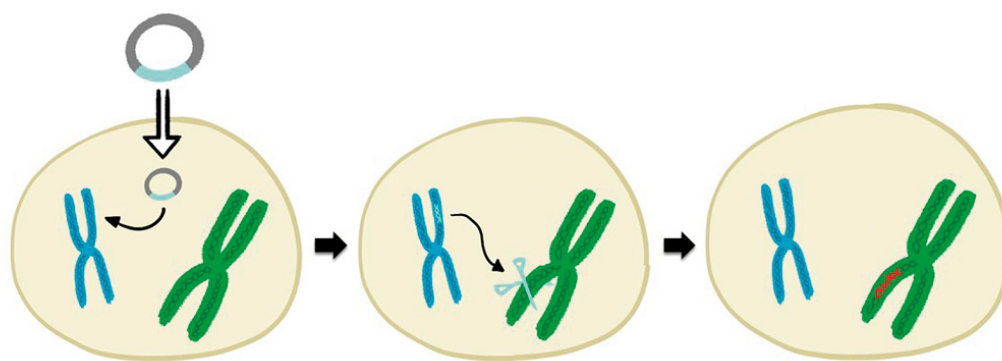


Figure 5: Applications of CRISPR/Cas, particularly in plants, are multistep processes, often including transgenesis (Old GE). First step: Non-targeted methods (such 'gene guns') are used to introduce the DNA for the nuclease into the cells. Only in the second step the nuclease can act in a targeted way.

This process may cause specific unintended genetic changes: for example, Yue et al. (2022), identified larger and smaller insertions as well as deletions caused by the biolistic method of gene insertion into papaya that were different to those caused by (non-regulated) conventional breeding methods or natural processes (see Chapter 5).

The multistep process can be varied in many details and adapted to specific needs and purposes. The results (also in terms of unintended effects) may depend on the individual researchers. As a result, each NGT application has a specific potential for intended or unintended genetic alterations in the genome. Therefore, in the same way that regulation has been established for transgenic GMOs, there are good reasons why, also in future, safety has to be assessed on a 'case-by-case' basis and in regard to specific 'events' that are characterized by the techniques applied, the steps of the process and the resulting intended and unintended effects (see also Chapter 5).

Further similarities between NGTs and older regulated GMOs (transgenic plants) can be seen in some of the traits which are under development. As shown in an overview produced by the Joint Research Center (JRC, 2021), herbicide resistant plants which were the first transgenic plants introduced into the markets, might re-enter the market as some of the first NGT-GMOs.

3. Overview of potential applications in agriculture and food production

There are several databases available which show a broad range of potential NGT applications in the context of agriculture and food production such as:

https://datam.jrc.ec.europa.eu/datam/mashup/NEW_GENOMIC_TECHNIQUES/index.html

<http://euginius.eu/euginius/pages/home.jsf>

<http://www.eu-sage.eu/genome-search>

These databases are based on different criteria and searches can bring different results: while JRC and Euginius list plants and animals, EU-SAGE only lists plants. JRC and EU-SAGE only address NGTs, Euginius also includes transgenic plants from 'Old GE' applications. There are further differences since JRC also includes medical applications, while EU SAGE and Euginius do not. While Euginius, at the end of July 2022, included 870 'GMOs', JRC refers to 645 organisms (426 plants) and EU SAGE to 546.

In general, the information made available in the databases on specific applications is of mixed quality and largely depends on the availability of relevant publications or entries in other databases. In several cases, the available information is poor, for example, because the data are considered to be confidential business information. No or only limited conclusions can be drawn from these data as to which of these applications will finally enter the market successfully.

However, if their limitations and specificity are taken into account, these databases can nevertheless be useful in that they provide an overview.

3.1 NGT applications in food plants

In regard to plants, the focus is on a broad range of species including cereals, oil and fiber crops, vegetables, fruit plants; trees and others are also being targeted in the research and development of NGT applications. See Figure 6 for a list (JRC database, July 2022).

Organism and species groups

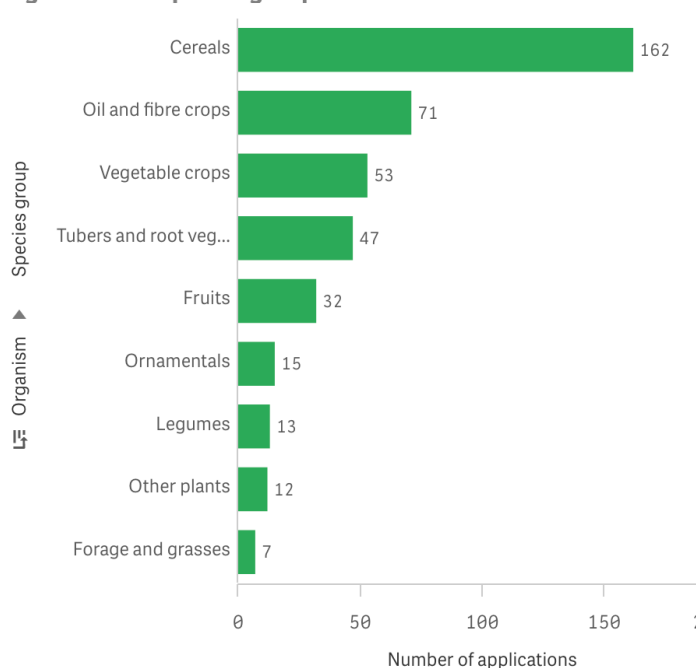


Figure 6: Plant species being used in NGT applications up until the end of July 2022. Source: https://datam.jrc.ec.europa.eu/datam/mashup/NEW_GENOMIC_TECHNIQUES/index.html

3. Overview of potential applications in agriculture and food production

In regard to potential traits, categories such as modified composition, stress tolerance (biotic and abiotic), yield, herbicide tolerance, storage performance and others are used. See Figure 7 for a list (JRC database, July 2022).

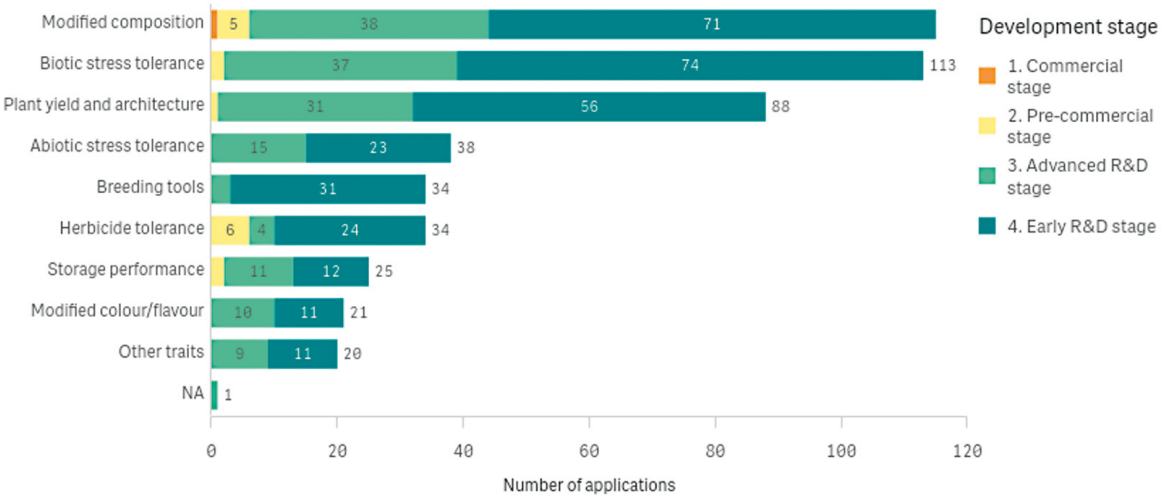


Figure 7: Traits that are assumed to be under development with the help of NGTs - up until the end of July 2022. Source: https://datam.jrc.ec.europa.eu/datam/mashup/NEW_GENOMIC_TECHNIQUES/index.html

Some indications as to potential products that may enter the market within the next few years can be derived from the JRC database (see Figure 8). Most of those applications concern herbicide-tolerant crops.

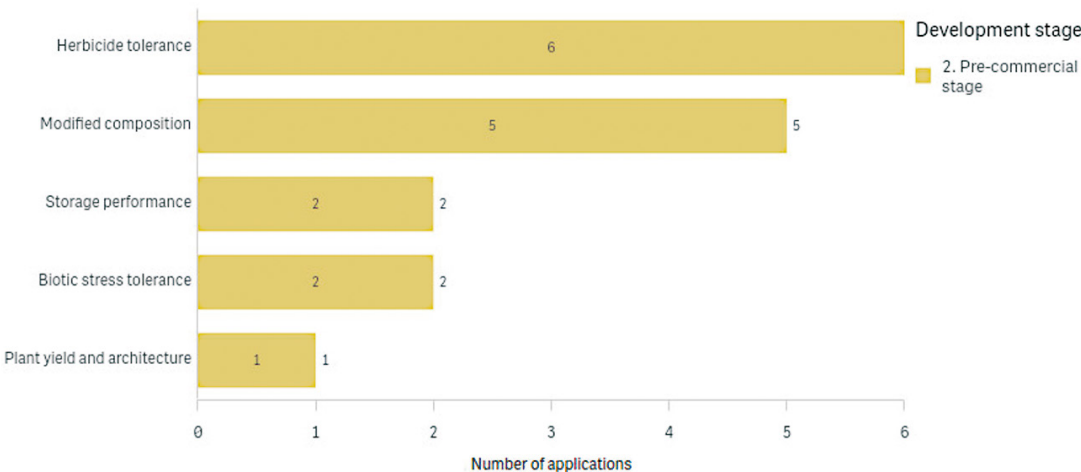


Figure 8: Traits that are claimed to be under development with the help of NGTs - up until the end of July 2022. Source: https://datam.jrc.ec.europa.eu/datam/mashup/NEW_GENOMIC_TECHNIQUES/index.html

3.2 NGT applications in animals

Most applications of NGTs in animals concern pharmaceutical research. There are, however, also several applications aimed at using animals in food production, such as cattle, pigs, poultry and fish. Again, data availability is in many cases poor. For example, no data are available for pigs listed in the Euginius database which were developed by the University of Edinburgh for disease resistance (GE-CD163 Pig), or developed by Revivicor with allergen reduction (GalSafe pig). A further example are hens developed by researchers in Israel which are supposed to not produce male offspring, where data only seem to be available from patent applications (such as WO2020178822) but not from any peer reviewed research. Some of the NGT animals are already approved for food production in the US (cattle) and Japan (fish) and are described below.

3.3 NGT applications involving microorganisms and viruses

Applications of NGTs may involve microorganisms such as bacteria, archaea, fungi, yeast, and in some cases, viruses. EFSA (EFSA, 2020b) published the results of a horizon scanning in 2020 which mentions more than 700 relevant publications, 45 cases and a selection of 11 examples. However, no clear distinction is made between synthetic biology and NGT. Starting with this overview, but also by taking into account other publications from ongoing horizon scans, we compiled the following, non-comprehensive list. Various NGTs were applied in these examples, some of them may also be considered to be synthetic biology (SynBio) (EFSA, 2020b). The applications are summarized as genetic engineering (GE) for greater clarity.

- Potential uses of GE microorganisms could include the engineering of ecosystems and microbial communities for purposes such as changing biodegradation, waste treatment and bioremediation (Mee et al., 2014; Qian et al., 2020; Wang et al., 2013).
- Several projects aim to change gut microbiota in animals and humans (Kim et al., 2018; Mimee et al., 2015; Ronda et al., 2019). Some of these approaches are under discussion for therapeutic concepts (Bober et al., 2018; Hwang & Chang, 2020; Mimee et al., 2016; Ozdemir, 2018; Sheth et al., 2016).
- Other applications directed at food and feed aim to change the composition of diets and products for human consumption (Lee et al., 2016; Mertens et al., 2019).
- GE applications to change gut microbiota are also under discussion, e.g. for insects such as flies (De Vooght et al., 2014; Gilbert et al., 2016) mosquitoes (Bilgo et al., 2017; Fang et al., 2011; Lovett et al., 2019; Ren et al., 2008) and bees (Leonard et al., 2018; Leonard et al., 2020; Rangberg et al., 2012). Some of these approaches are known as 'paratransgenesis', which means that the biological characteristics of the target host are changed by genetically engineering its symbiotic bacteria, for example, to eliminate a pathogen from insects via the expression of effector molecules (Wilke et al., 2015).
- Similar approaches are under discussion for corals (Levin et al., 2017).
- In agriculture, there are ongoing discussions in regard to applications to change the microbiomes of plants, e.g. mycorrhiza or endophytes (Arif et al., 2020; Checcucci et al., 2018; Hettiarachchige et al., 2019; Ke et al., 2022; Vorholt et al., 2017).
- In agriculture, GE applications targeting soil microorganisms are also being discussed (Shelake et al., 2019; Shulse et al., 2019; Temme et al., 2012).
- Further potential uses include the usage of GE microorganisms as pesticides (Azizoglu et al., 2020; Fang et al., 2014; Leclère et al., 2005; Scheepmaker et al., 2016; Tseng et al., 2005; Wang et al., 2011).

- Several projects are looking at using GE microorganisms (such as cyanobacteria or algae) in energy production (Motomura et al., 2018; Nozzi et al., 2013; Wang et al., 2013).
- Other applications include viral systems, such as bacteriophages (Citorik et al., 2014; Lemire et al., 2018), and even the dissemination of genetically engineered/GE viruses via insects ('insect allies') for potential military purposes (Lentzos et al., 2022; Reeves et al., 2018).

3.4 NGT plants and animals already introduced into markets outside the EU

In the European Union, NGT-GMOs are regulated under the GMO legislation. Products obtained through NGTs are not currently marketed in the EU. However, the Euginius database (July 2022) lists three animals for food production and two plants which already have market approval in Japan and the US:

- The US FDA (Food and Drug Administration) published its opinion in 2022 on cattle with short, slick coats (meant to be beneficial in higher temperatures), expressing no objections against the marketing of products derived from NGT beef cattle and their progeny.
- Two NGT fish 'events', sea bream and pufferfish, were approved for sale in Japan in 2021. The fish were developed for faster growth and a higher proportion of muscle or a larger body size compared to conventional fish.
- Japan approved the commercial sale of an NGT tomato producing a higher amount of GABA (γ-Aminobutyric acid) in 2021. The fruits are supposed to reduce blood pressure if consumed.
- Calyxt was the first company to bring seeds derived from NGTs to the US market. The soybean with high-oleic acid oil content was brought to market in the US in 2019. However, the soy failed to produce the desired yields for the farmers and did not meet the expectations of the investors. It appears that the genetic intervention actually resulted in a reduced soybean harvest. Consequently, the company producing the soybean, Calyxt, exited this line of business in 2020. Sales, earnings and the value of Calyxt stock fell dramatically as a result. It appears to be doubtful whether the soybean is actually still on the market.⁴

4 <https://www.bizjournals.com/twincities/news/2022/09/22/calyxt-considering-sale-of-assets-merger.html>

4. Technical and biological characteristics of selected examples

The following section of the report contains some selected examples with relevance to the questions raised in the TOR that are explained in more detail. These examples include plants and animals recently given market approval in the US and Japan.

In addition, some further cases were selected for the following reasons:

- NGT mushrooms were the first ‘CRISPR-food’ product meant for the US market.
- Hornless cattle were the first NGT product to be withdrawn from market application in the US (and Brazil).
- Herbicide-resistant maize is the first NGT plant for which an application has been filed for market approval in the EU.
- CRISPR laying hens – there are ongoing discussions about whether they should be allowed in the EU without a mandatory approval process.
- NGT camelina was chosen because of its relevance to both environmental and health risks.
- The examples of wheat and tomatoes were chosen because of their relevance for food production and some specific comments made by EFSA.

Since the TOR included in this report deal with questions of regulation, the examples listed above will also be used to exemplify some of these aspects.

4.1 Examples of NGT plants and mushrooms

The following section contains short technical case studies describing examples of NGT plants for use in food production. Some of them have already been applied for and/or have marketing approval.

4.1.1 CRISPR-mushrooms

This example was the first NGT food product derived from CRISPR/Cas (SDN-1) that was declared to be safe by US APHIS (Animal and Plant Health Inspection Service) and ready for market introduction in 2016 (Waltz, 2016). However, the CRISPR/Cas mushrooms are so far not available to consumers. Edible mushrooms were created using CRISPR/Cas to stop cut surfaces from turning brown by blocking the function of the polyphenol oxidase gene; the non-browning mushrooms were meant to have a longer storage and shelf-life. This was achieved by destroying the structure of the target gene that is present in the fungus in several copies, and meant that the fungus was changed in several locations on the same gene. Such a pattern of genetic change is unlikely to appear spontaneously. The responsible US authority, APHIS, approved the mushrooms in April 2016,⁵ because it was, in their view, sufficient that the developers said that no additional DNA had been inserted. At this stage, no further investigations were required to check whether other substances in the mushrooms had changed. No data on unwanted changes in the genome were available. It seems there is also no peer reviewed scientific publication on how exactly the properties of these mushrooms were intentionally or unintentionally changed. The likelihood of these mushrooms ever really being brought to market still seems not decided.

⁵ www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regulated/Regulated_Article_Letters_of_Inquiry

4.1.2 Herbicide-resistant maize

The first NGT plant for which an application for market approval was sought in the EU is a maize variety developed by Pioneer/Corteva (previously owned by DowDuPont).⁶ The plant was generated with the help of CRISPR/Cas (SDN-3). Maize DP915635 is resistant to the herbicide glufosinate and produces an insecticidal toxin found in specific ferns growing on trees.⁷ The maize was generated with a combination of old and new genetic engineering methods: to deliver the CRISPR/Cas ‘gene scissor’ into the plant cells, they are first bombarded with small particles (‘gene gun’). The cells then produce the enzyme for the gene scissors which is subsequently inserted as a DNA-sequence into the maize genome. This additional DNA-sequence is meant to facilitate the insertion of other genes, and is therefore known as a ‘landing pad’. In the next step, a further gene construct is inserted into the ‘landing pad’ in the maize genome, thus conferring resistance to the herbicide and producing the fern toxin. The company has filed several patent applications for the plants, some of which have already been granted in Europe.

4.1.3 GABA tomato

Japan approved the first NGT plants for consumption in Japan in 2021.⁸ These are tomatoes with a much higher concentration of a specific plant compound (GABA) compared to conventionally bred tomatoes. Several previous attempts to achieve a permanently higher level of GABA in the plants through conventional breeding failed. GABA (γ-Aminobutyric acid) is an inhibitory neurotransmitter in the central nervous system which may, amongst others, reduce blood pressure. The tomatoes will therefore be introduced as a modern ‘lifestyle’ product. At the same time, it is known that GABA has a multifunctional role in tomato plants: it influences, for instance, plant growth, resistance to plants pests and diseases as well as several other metabolic reactions. Due to the multifunctional role of GABA, it has to be assumed that the genetic intervention will affect plant metabolism on several levels. These changes can also cause unintended health effects at the stage of consumption. In addition, the plants can show unexpected reactions to environmental stress conditions, which can again have an impact on the safety of food products (Nonaka et al., 2017). As far as is known, no data are available on the potential benefits or on potential adverse effects.

4.1.4 CRISPR-camelina

Many scientists in the US and the EU are interested in genetically engineering camelina (*Camelina sativa*). One focus is on the production of agro-fuel. *Camelina* plants in which 18 sites on the genome were changed using CRISPR/Cas gene-scissors were developed in the US (Morineau et al., 2017). The multistep process also involved the application of ‘old’ non-targeted methods of genetic engineering known as transformation by *Agrobacterium tumefaciens*. Since *C. sativa* is an allohexaploid plant composed of three sub-genomes, conventional breeding faces substantial limitations where homozygous mutations of homeologous genes is required. As a result, the NGT plants show patterns of genetic change and altered oil quality that would not be possible or at least very unlikely to achieve with conventional breeding even without inserting additional genes. In 2018, APHIS declared the plants to be safe for the market.⁹ *Camelina* is one of the oldest cultivated plants in Europe and is an important plant species for pollinating insects. The plants can survive and multiply in the

6 www.testbiotech.org/pressemitteilung/erster-zulassungsantrag-fuer-crispr-pflanzen-in-eu

7 www.testbiotech.org/content/application-authorisation-maize-dp915635-pioneer

8 <http://euginius.eu/euginius/pages/home.jsf>

9 www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regulated/Regulated_Article_Letters_of_Inquiry

environment as well as cross into natural populations. Experts are warning that risks can arise from the cultivation of the genetically modified plants due to their altered oil quality and potential uncontrolled spread (see Kawall, 2021a). For example, the oleic acids formed in genetically modified plants can change the growth and reproductive rate of wild animals feeding on them. Problems could also arise if the oil seeds are accidentally introduced into food and feed.

4.1.5 De-novo domesticated tomato

In 2018, researchers succeeded in using CRISPR/Cas to change several genes at the same time in non-domesticated wild tomatoes. Six genes were knocked out with the result that small fruits growing on bushy plants were changed into tomatoes that look similar to the ones currently being marketed (Zsögön et al., 2018). This was intended to show that the outcomes of years of conventional breeding are replicable within a very short period of time using NGTs. Even though no additional genes were inserted, the impact was extraordinary: the number of fruits, their size, form and composition, as well as the architecture of the plants, were changed in just a few working steps and within a short period of time. EFSA (2022b) also analyzed this NGT plant.

4.1.6 Wheat 'events'

The following section contains four examples taken from species of wheat (*Triticum aestivum*) with different traits. They were all developed with NGTs (SDN-1) using multistep processes (involving old GE such as biolistic methods). Bread wheat (*Triticum aestivum*) is characterized by its huge genome, comprising six sets of chromosomes (Guan et al., 2020). This causes some difficulties in conventional breeding since in many cases a high number of gene duplications are involved in a specific trait.

As explained above, NGTs now appear to offer the potential to overcome the limitations of previous breeding methods by introducing genetic changes in all gene copies at the same time. However, in the case of the selected NGT traits, there are also reasons to assume that the intended (on-target) genetic alterations are associated with unintended biological characteristics (see below).

Trait 1 - reduction in gluten: Gluten proteins in wheat are thought to trigger several gluten-related disorders, including celiac disease (Gatti et al., 2020). It is known that alpha-gliadin peptides contribute to the overall concentration of gluten in bakery products (Verma et al., 2021). These genes occur within a large family of genes that are present in multiple copies at different locations in the genome. With the help of the CRISPR/Cas nuclease, scientists succeeded in 2018 in switching off a large number of these genes: 35 of 45 genes that are necessary to produce alpha-gliadins were knocked out (Sanchez-Leon et al., 2018). This resulted in a new genotype and also resulted in a greater degree of complexity for risk assessment (EFSA, 2021). However, gliadins are, for example, also known to play an important role in the plant responses to stress conditions, including drought and heat (Blumenthal et al., 1995; Marín-Sanz et al., 2022; Phakela et al., 2021). Therefore, larger reductions in the content of alpha-gliadins may also unintentionally impact the heat and/or drought tolerance of this trait.

Trait 2 - reduction in acrylamide: CRISPR/Cas9 was used to reduce the content of the free amino acid asparagine in wheat (Raffan et al., 2021). Free asparagine is present in higher concentrations in wheat grain. It is a precursor of acrylamide, which forms during the baking, toasting and high temperature processing of foods made from wheat. Acrylamide has been shown to have carcinogenic properties. The relevant gene (asn2) occurs a total of six times in the wheat genome. In some of the NGT wheat plants, the asparagine content was reduced by 90% compared to the wild type. Other methods have not previously achieved such a strong reduction

of the asparagine content in wheat grain. While it seems that a gene function involved in the production of the amino acid asparagine was to some extent successfully blocked, this also creates problems since asparagine is also involved in seed germination, plant growth, stress response and defense mechanisms. It was found that some lines of this CRISPR-wheat almost lost capacity to germinate (Raffan et al., 2021). This wheat is about to be tested in field trials in the UK¹⁰.

Trait 3 - reduction in susceptibility to powdery mildew: The mildew resistance locus o (mlo) gene in barley is of interest for several projects. There are three different mlo genes involved in resistance to powdery mildew which is found in natural populations. One of the studies used TALENS to target the mlo gene in hexaploid wheat (Wang et al., 2014). The nuclease introduced alterations in all three homoeoalleles of mlo in wheat, enabling their parallel knock-out. This was not previously possible with either chemical mutagenesis or other breeding methods. The simultaneous knock-out of the three homoeoalleles conferred a broad-spectrum resistance to powdery mildew in these lines. In addition, unintended effects were described in the wheat (i.e. leaf chlorosis under growth conditions), which were also not observed in randomly mutated plants (Acevedo-Garcia et al., 2017). Growth aberration, accelerated senescence, induced necrosis, increased susceptibility to other fungal pathogens are all unintended effects described in the context of this NGT trait – which may, however, also be overcome (Spanu, 2022).

Trait 4 - increased immune response to fungal diseases: In the German PILTON¹¹ project, researchers are aiming to block the gene function of a gene (CPL3) in wheat that is known as a regulator in the fine tuning of immune responses in the plants (Koiwa et al., 2002; Li et al., 2014). The intention is to block the function of the CPL3 gene by using the CRISPR/Cpf1 nuclease variant. The plant might thus be able to prolong or enhance its immune response to plant diseases, such as wheat leaf rust (*Puccinia tritica*), which is a fungal disease affecting leaves and grains. With the help of Cpf1, it may be possible to knock out all gene copies on each of the six sets of chromosomes. However, as preliminary results show, the loss of the gene function is associated with fitness costs for the plants: they are likely to show slower growth and earlier flowering which indicates reduced fitness. The start of the project has already been announced, the initial data were meant to be published in 2021, however, as of August 2022, it seems no results have been published yet.

4.2 Examples of NGT animals

The following section of the report contains short technical case studies of NGT animals for food production that were either approved for the market or for which applications have been filed.

4.2.1 Cattle with short, slick coats

This is the first NGT animal for food production deregulated in the US¹², but not yet on the market. In March 2022, the US FDA has decided to issue approval for CRISPR/Cas cattle with short, slick coats for agricultural purposes.¹³ CRISPR/Cas was used to alter the genes of a receptor for the hormone prolactin (SDN-1). The aim was to generate cattle with shorter hair, a trait called SLICK which is already known from traditional breeding. Animals with this conventionally bred trait are, according to various studies, better able to cope with higher ambient temperatures (see, for example, Hansen, 2020). Four calves were examined, one of which was not

10 <https://www.gov.uk/government/publications/genetically-modified-organisms-rothamsted-research-21r0801>

11 <https://pilton.bdp-online.de/?lang=en>

12 <http://euginius.eu/euginius/pages/home.jsf>

13 <https://cacmap.fda.gov/media/155706/download>

genetically engineered, probably because the gene scissors had failed to work as expected. Another calf died unexpectedly, but the FDA assumes that this incident was not related to the genetic intervention. It is remarkable that neither of the ‘successfully’ genetically engineered animals show the intended changes consistently in all the cells of their body. This phenomenon is known as genetic mosaicism or chimeric formation. Unintended genetic changes were also found in the cattle, these were, however, considered to be less severe. At the same time, the data provided by the FDA includes no proof of whether the animals will stay healthy over their lifetime. If the male animals are used for further breeding, their intended and unintended genetic changes could rapidly spread throughout larger cattle populations. The animals will be marketed by Recombinetics and its affiliated company, Acceligen, also has filed patents (WO2017053315).

4.2.2 Hornless NGT cattle

This is the first NGT animal for which application was withdrawn from US market and also Brazil. In 2019, the US FDA scrutinized and rejected the approval of NGT hornless cattle engineered with TALEN gene scissors (SDN-2). At that time, it was shown that the processes of genetic engineering had caused genes from bacteria to be unintentionally integrated into the genome of the cattle and passed on to the next generation (Norris et al., 2020). The cattle had been genetically engineered before 2016 (Carlson et al., 2016), but it was only in 2019 that scientists noticed that genetic material of the bacteria used in the process had also been introduced into the genome of the cattle (Norris et al., 2020). Amongst other things, they found complete DNA-fragments able to confer resistance to antibiotics in the genomes. If the genetically engineered cattle had been used for breeding as planned, the unwanted genes could have spread rapidly through dairy herds. Consequently, the NGT cattle were not approved for the market and had to be slaughtered. In Brazil, the cattle already passed deregulation, were withdrawn after the findings of Norris et al. (2020).¹⁴ Hornless cattle were also generated using CRISPR/Cas (SDN-2). The process of the NGT similarly caused many unintended effects (Schuster et al., 2020). In 2022, another study was published (Hennig et al., 2022) in which researchers tried to apply CRISPR/Cas to delete a targeted region in the genome instead of inserting a new gene function. While the deletion was partially successful, all calves still developed horn buds.

4.2.3 NGT seabream with a change in growth

Japan allowed the first NGT fish to be marketed in 2021. They were produced with the help of CRISPR/Cas (SDN-1)¹⁵. Gene functions which regulate muscle growth were blocked in the genome of red seabream (*Pagrus major*). In response, the fish had more muscle growth, a larger body size, a reduction in body length and an abnormal position of the vertebra (Kishimoto et al., 2018). In comparison to the wild type, the fish gain weight faster and seem to move slower. No data are available to show how the genetic alteration affects their life span or health in general. There are also apparently no data available on animal welfare. There are similarly no data on changes in the composition of flesh in the NGT fish or any potential impact on consumers. On a technical level, this shows that the genetic intervention was not precise: starting with hundreds of GE fish, the researchers selected those deemed suitable for further breeding. The targeted gene sites showed differing alterations. Furthermore, in many cases, genes were altered in some organs, but not in all cells of the body. It is assumed that the cost of feeding GE fish reared in special containers could be reduced (Kishimoto et al., 2018).

¹⁴ <http://ctnbio.mctic.gov.br/tecnologias-inovadoras-de-melhoramento-genetico-rn16->

¹⁵ <http://euginius.eu/euginius/pages/home.jsf>

4.2.4 NGT pufferfish with a change in growth

Japan approved another CRISPR/Cas (SDN-1) NGT fish for the market in 2021¹⁶. Gene functions were blocked in the genome of pufferfish (*Takifugu rubripes*) that control the appetite of the fish: the leptin receptor gene in the fish was disrupted, which may be associated with weight gain and diabetes-like symptoms (Kurokawa & Murashita, 2009). Until now, fish species such as zebra fish (*Danio rerio*) inheriting similar genetic defects have been used as disease models to explore complex metabolic disorders in mammals (Audira et al., 2018). There are further studies on medaka fish (*Oryzias latipes*) which showed large deposits of visceral fat in the adult fish (Chisada et al., 2014). However, it is not possible to compare these data with the NGT pufferfish since peer reviewed publications seem to be missing. It seems that the cost of feeding of the NGT fish reared in special containers may be reduced. At least, this the rationale behind the filed patent applications (such as WO2019066052) for the industrial usage of the fish.

4.2.5 NGT hens

Researchers in Israel have used CRISPR/Cas to alter hens so that no male offspring are able to hatch. A deadly gene is passed on to any male offspring, and is intended to kill the male embryos in the egg before they hatch. At the same time, the female offspring will supposedly develop normally so that they can be used as laying hens for egg production. This NGT application aims to solve the problem of male offspring in the process of breeding hens, as these are killed after hatching because they are of no economic benefit to the food producers. Patents for the process and the resulting hens have already been filed (such as WO2020178822) and could in due course be marketed in cooperation with a US company. The patent applicants claim that their technology is safe and there are no transgenes in the genome of the laying hens. However, no peer reviewed data could be identified on the intended and unintended effects in NGT poultry and their eggs.¹⁷

¹⁶ https://euginius.eu/euginius/pages/gmo_detail.jsf?gmoname=GE-lepr+tiger+pufferfish

¹⁷ For further information also see: <https://www.testbiotech.org/en/news/new-ge-deregulated-through-backdoor>

5. Issues with relevance to the risk assessment of NGT-GMOs

The following section provides an overview of some categories of environmental hazards and risks associated with NGTs. In addition, selected applications exemplify hazards and risks. These show that the technical potential of NGTs and their risks and hazards are closely interrelated.

5.1 Specific risks associated with NGT plants

As shown, NGTs can be used to achieve genomic changes extending beyond what is known from conventional breeding, even if no additional genes are inserted. Compared to methods of conventional breeding (including random mutagenesis), NGTs can overcome the boundaries of natural genome organization that have emerged over the course of evolution. CRISPR/Cas ‘gene scissors’ in particular make it possible to alter the genome to a much greater extent than with any previous breeding.

The greater accessibility of the genome enables pervasive changes in the biological characteristics of the organisms, even without the insertion of additional genes; and also enables more extreme versions of already known traits or the generation of new traits which are often associated with ‘trade-off’ responses (side effects).

Furthermore, unintended genetic changes have been observed (on-target and off-target) that are specific to the processes of NGTs and unlikely to occur due to random processes or conventional breeding. These genetic irregularities must be considered to be risks inherent to the technology.

Risk assessment needs to consider both the indirect effects caused by the intended traits and the unintended genetic alterations (see also Figure1).

5.1.1 Risks associated with the intentionally introduced traits

Many of the intended NGT traits that can be generated without the insertion of any new gene functions (SDN-I processes), such as changes in oil content (Morineau et al., 2017), protein composition (Sanchez-Leon et al., 2018), sugar concentration (Kannan et al., 2018), plant architecture (Shen et al., 2017), yield (Roldan et al., 2017) or biologically active plant constituents such as GABA (Nonaka, et al., 2017), reach beyond what is likely to be achieved by conventional breeding (for overview, also see Kawall, 2021b). These new intended GE traits are the result of specific patterns of genetic changes introduced by gene scissors such as CRISPR/Cas. Similarly to transgenic plants that produce insecticidal proteins originating from bacteria, such genotypes are unlikely to result from random mutations and other conventional breeding methods. The depth of interventions may unavoidably cause ‘trade-off’ responses (metabolic side effects) in the organisms which are associated with the unintended biological effects. The following section describes the risks that can emerge from these genotypes.

a) Case study - NGT camelina

A first detailed risk scenario for NGT plants was provided by Kawall (2021a). This scenario examined NGT camelina with intended changes in oil content that are unlikely to be achievable with conventional breeding (Morineau et al., 2017, see also example 4.1.4). Kawall (2021a) shows that if the composition of the fatty acids is changed, unintended effects on various processes can occur in addition to the desired properties. This may be related to effects on the formation of certain messenger substances with which plants communicate and with which they, for example, ‘warn’ of a pest infestation. A change in the composition of fatty acids can affect and influence existing food webs. In addition, there is also the possibility that genome-edited plants will hybridize with wild species leading to unintended effects in subsequent generations. At the same time, the genome-edited camelina has the potential to persist in the environment and spread uncontrollably. Therefore, the risks

identified concern the food web, the defense mechanisms of the plants and uncontrolled gene flow. Kawall (2021a) concludes: *“There are also special concerns regarding interventions in well-balanced signalling pathways that regulate communication and interactions between plants, animals, associated microbiomes, beneficial predators and pollinators potentially affecting ecoservices. In addition, next-generation effects can occur in case genome-edited plants have the potential to persist and propagate in the environment.”*

b) Case study - NGT wheat

As shown in example 4.1.6, there are several NGT applications in wheat which result in genotypes that are unlikely to result from the use of previous breeding methods. EFSA analyzed one of these examples EFSA (2021) when discussing new challenges for risk assessment (Sanchez-Leon 2018, see also trait 1 of example 4.1.6). EFSA (2021) states in its case study: *“(…) the large number of mutations required to achieve gluten-free wheat is far beyond any plant previously assessed. This is likely to require SynBio approaches to correctly identify all gliadins and glutenins in the hexaploid genome of bread wheat and to identify an engineering strategy that introduced mutations of the correct nature and positions in each gene to prevent the accumulation of any peptide fragments associated with initiation of the inflammatory cascade”*. Kawall (2021b) summarized these findings: *“One example to illustrate the generic risks of CRISPR/Cas is a wheat generated by Sanchez-Leon et al. (...). The same study was also listed as an example by the European Food Safety Authority (EFSA) in its recent scientific opinion (...) According to EFSA, their case study shows that a strategy is needed to identify the type of alteration and position in each individual gene to prevent the accumulation of any unintended peptide fragments. Such analyses are of major importance for risk assessment, especially when considering SDN-1 applications with a higher level of complexity and/or depth of intervention.”*

In conclusion, this case shows that even if changes are successfully introduced into the target genes, complex questions in regard to the safety of the plants need to be considered: each targeted genetic site needs to undergo a detailed examination to determine whether the alpha-gliadin proteins are still being produced, or if new proteins are produced unintentionally, or if any other unintended effects may occur.

c) Case study - NGT de-novo domesticated tomato

Zsögön et al., (2018), Kawall (2021b) and EFSA (2022d) appear to come to similar conclusions for de novo domesticated tomatoes (see also example 4.1.5). As Kawall (2021b), states: *“(…) plants altered with SDN-1 which contain traits that are known from cultivated varieties, but are expressed in a new genetic background, cannot be equated to their conventional or natural counterparts, as the corresponding target gene(s) might have divergent functions or interactions in different species. De novo domesticated plants generated using CRISPR/Cas9 are interesting examples in that regard. (...) Comprehensive environmental and health risk assessments will be needed to ensure that no effects with negative impacts have occurred.”*

EFSA (2022d) comes to the conclusion that current EU guidance, which is based on comparative risk assessment, would not be sufficient to assess these risks: *“This case study highlighted potential issues for the applicability of the existing comparative analysis guidelines with respect to the availability of the conventional counterpart and non-GM reference varieties. The parental line used to obtain this SynBio product (S. pimpinellifolium) is not commonly consumed (...). The selection of reference varieties would also be challenging: wild tomato varieties of commercial use as food and feed might not be available. Tomatoes cultivated for food and feed purposes could be of interest for comparison, considering the intended use of the SynBio tomato, but would be genetically far from the SynBio plant. As a consequence of the lack of an appropriate comparator (...), the comparative analysis for this SynBio case may not be carried out as described in the existing guidelines.”*

In conclusion, this case seems to exemplify a specific aspect of the unique technical potential of CRISPR/Cas: until now, traditional breeding has developed new varieties step-by-step over many years. Now, however, CRISPR/Cas can change multiple copies of a gene as well as change several different genes at the same time in just one step, an approach known as ‘multiplexing’ (Kawall et al., 2020; Raitskin and Patron, 2016). Even though no additional genes are inserted, the impact is extraordinary: the number of fruits, their size, form and compounds as well as the architecture of the plants can be changed in just a few working steps and within a short period of time. However, the resulting risks are complex. Whether these tomatoes, which look just like normal tomatoes, are actually safe to eat can only be clarified by thorough investigations.

d) Overview: Unintended effects linked to intended changes

In general, direct and indirect effects can be caused by the intentionally generated traits. The traits derived from NGT can cause extreme variants of biological characteristics and also generate new traits which are unlikely to be achieved with conventional breeding. The depth of intervention may unavoidably cause ‘trade-off’ responses (metabolic side effects) in the organisms. The traits derived from NGTs can likewise generate extreme variants of biological characteristics and new traits which are unlikely to be achieved with conventional breeding. The unintended direct and indirect effects associated with the intended traits may, for example, have serious adverse impacts on the environment, plant or animal health, agricultural yield, pesticide use and food safety. If released into the environment, the interactions with other NGT-GMOs and with the environment, including pests, pathogens, climatic conditions etc., adds further complexity to these risk scenarios.

In many cases, the desired advantages are linked to trade-offs caused by the pervasive changes in biological characteristics. As the summary of examples in Table 1 shows, such unintended effects were identified as relevant to several NGT-plants. Similarly, as is the case with the intended traits, these unintended effects are likely to go beyond what was caused by previous methods of breeding (see Kawall, 2021a/b).

Table 1: Selected examples of unintended effects associated with the intended traits and relevant to the risk assessment of NGT plants.

Species	Intended trait	Unintended metabolic and physiological effects and hypothesized risks
Wheat	Powdery mildew resistance (example 4.1.6, trait 3)	Growth aberration, accelerated senescence, induced necrosis, increased susceptibility to other fungal pathogens. (Spanu, 2022)
Wheat	Decreased acrylamide content (example 4.1.6, trait 2)	Reduced growth and germination rate, potentially increased susceptibility to fungal plant pathogens. (Raffan et al., 2021)
<i>Camelina</i>	Altered oil quality (example 4.1.4)	Weakened defense mechanisms against biotic (pathogens) or abiotic (climate change) stressors. (Kawall, 2021)
Tomato	Enhanced GABA content (example 4.1.3)	The changes in plant composition may also cause unintended health effects at the stage of consumption. Furthermore, unexpected reactions of the plants to environmental stress conditions are not unlikely. (Nonaka et al., 2017)
Tomato	Accelerated domestication (example 4.1.5)	Differences in plant composition are observed in comparison to previously bred tomatoes. These differences may also impact health at the stage of consumption. (Zsögön et al., 2018)
Rice	Improved salinity tolerance	Enhanced invasiveness might occur in weedy rice after hybridization. (Zhang et al., 2019)

Unintended effects associated with the intended traits listed in Table 1 may have serious adverse impacts on the environment, plant health, agricultural yield, pesticide use, and/or food safety. If grown in fields, the interactions between NGT-GMOs and the environment, including pests, pathogens, climatic conditions etc., adds further complexity to these risks. These unintended direct or indirect effects associated with the intended trait are the result of interactions in the complex networks of genes, proteins and other biologically active molecules. Such unintended effects can also emerge in cases where the genetic intervention is targeted and precise.

5.1.2 Specific, unintended effects caused by the processes of NGTs

Similarly to the intended traits, unintended effects can also cause patterns of genetic change that go beyond what can be achieved with conventional breeding and result in specific risks. The unintended genetic changes include off-target DNA cleavage, repetitive unit deletion, indels of various sizes, larger structural changes in the targeted genomic region and the unintended insertion of transgenes. While some of these 'types' of genetic alteration might also be observed in conventional breeding (EFSA, 2022f), the probability for these changes to occur on a specific site in the genome and the resulting genotype can be very different (for overview see Kawall, 2021). If these unintended effects are overlooked, they may quickly spread within large populations. Moreover, if the seeds are used for further propagation and breeding, potentially hazardous genetic alterations can remain undetected for a longer period of time and may also accumulate.

Findings relating to a broad range of unintended effects caused by CRISPR/Cas have already been published. Several publications describe how CRISPR/Cas causes unintended changes, including off-target effects, on-target effects and chromosomal rearrangements (Adikusuma et al., 2018; Biswas et al., 2020; Burgio et al., 2020; Cho et al., 2014; Grunewald et al., 2019; Haapaniemi et al., 2018; Kapahnke et al., 2016; Kosicki et al., 2018; Kosicki et al., 2020; Lalonde et al., 2017; Leibowitz et al., 2020; Liu et al., 2021; Michno et al., 2020; Ono et al., 2019; Sharpe, 2017; Skryabin et al., 2020; Tuladhar et al., 2019; Weisheit et al. 2020; Wolt et al., 2016).

In several cases, unintended genetic alterations in the target region (on-target) or in other genomic regions (off-target) specific to gene scissors, such as CRISPR/Cas, have been described. For example, larger structural genomic changes, such as translocations, deletions, duplications, inversions and scrambling of chromosomal sequences, can occur near the SDN target site (as well as at the SDN target site) which would otherwise be unlikely to occur (see e.g., Hahn & Nekrasov 2019). In addition, specific unintended on-target effects often include the integration of DNA from vector DNA derived from transformation processes, where, for example, bacterial DNA was unexpectedly integrated (e.g. Andersson et al., 2017; Li et al., 2015; Zhang et al., 2018). Overall, the CRISPR/Cas9 system has been confirmed to have a high frequency of integration into the target site, resulting in large deletions at the target sites (Lee et al., 2019; Yang et al., 2022).

In general, the CRISPR/Cas machinery is known for its potential to confuse target regions with specific off-target regions, in addition to causing the unintended insertion of additional genes, decoupling of genes and other specific genomic alterations (of categories such as inversions, deletions or rearrangements) that are unlikely to emerge from spontaneous mutations or physical and chemical mutagenesis (see, for example, Biswas et al., 2020 Braatz et al., 2017; Hahn & Nekrasov 2019). In some cases, unusual patterns of inheritance have also been observed, thus escaping the Mendelian rules (Yang, et al., 2022).

These unintended changes can cause a variety of unwanted effects. For example, the integrity of a non-target gene may be compromised if its coding region is cleaved by CRISPR/Cas (e.g. cleavage at off-target-sites). This could lead to changes in the metabolism of the organism that could affect its safety for human health and

the environment. Such effects are highly dependent on the genomic context within which such unintended alterations occur (e.g. within a gene, loss of function mutations; outside of genes, unintended alterations in promoters could alter gene expression).

As a result, similar to the case with the intended effects, unintended effects can also cause patterns of genetic change that go beyond what can be achieved with conventional breeding and result in specific risks. Yang et al. (2022) give an overview of irregular genetic changes and specific unintended effects caused by intrinsic factors of the CRISPR/Cas systems in plants. These include off-target DNA cleavage, repetitive unit deletion, and indels of various sizes (Chakrabarti et al., 2019; Kapusi et al., 2017; Manghwar et al. 2020; Molla and Yang, 2020; Zhang et al., 2014). In this context, the dosage of CRISPR/Cas complexes expressed in cells can also result in a significant increase of off-target mutation frequency (Ordon et al., 2017; Zhang et al., 2018).

In addition, it has to be taken into account that New GE is a multi-step process, with inherent and specific risks independent of the purposed traits. For example, NGTs such as CRISPR/Cas applied in plants, typically make use of older genetic engineering ('Old GE') methods, i.e. non-targeted methods to deliver the DNA coding for the nuclease into the cells. Thus, in most cases, the result of the first step of the CRISPR/Cas application is a transgenic plant which may show a broad range of unintended genetic changes that are unlikely to emerge from conventional breeding. Conventional breeding is only used at the end of the multistep process to remove the transgenic elements from the plant genome (segregation breeding). However, without adequate standards of risk assessment in place, the unintended genetic changes may remain undetected in the genome, spread quickly and widely within the populations and may also accumulate.

The mechanisms and outcomes of these technical processes for the insertion of genes, such as biolistic methods and usage of *Agrobacterium tumefaciens*, cannot be equated to effects occurring naturally or in previous methods of breeding. For example, Yue et al. (2022) identified larger and smaller insertions as well as deletions caused by the biolistic method of gene insertion into papaya. The larger insertion consisted of 77 rearranged and translocated fragments, the larger deletion included 44 genes. More than 600 genes were changed in their activity. The changes caused by the method of genetic engineering could be clearly distinguished from other genomic changes, which had occurred during the (around) 4000 years of the domestication of papayas. In conclusion, the processes used for the technical insertion of DNA can cause effects which are different in their scale, in the sites and in the patterns of the genetic change as well as their biological characteristics when compared to those of non-regulated breeding methods or natural processes. This is also true even if no additional genetic information is added to the gene pool of a species. Such effects may be related to epigenetic regulation, the disruption of genes, position effects, open reading frames, the unintended introduction of additional genes, changes in gene expression and genomic interactions which can involve plant constituents, plant composition and agronomic characteristics (Forsbach et al., 2003; Gelvin et al., 2017; Jupe et al., 2019; Makarevitch et al., 2003; Liu et al., 2019; Rang et al., 2005; Windels et al., 2003; Yue et al., 2022).

In summary, at each stage of the process - including (i) insertion of the gene scissor DNA into the cells, (ii) target gene recognition and cutting and (iii) cellular repair of the genes - specific unintended alterations can occur along with risks (see Figure 1). Some of the unintended genetic alterations caused by CRISPR/Cas (on-target and off-target) are summarized in Figure 9.

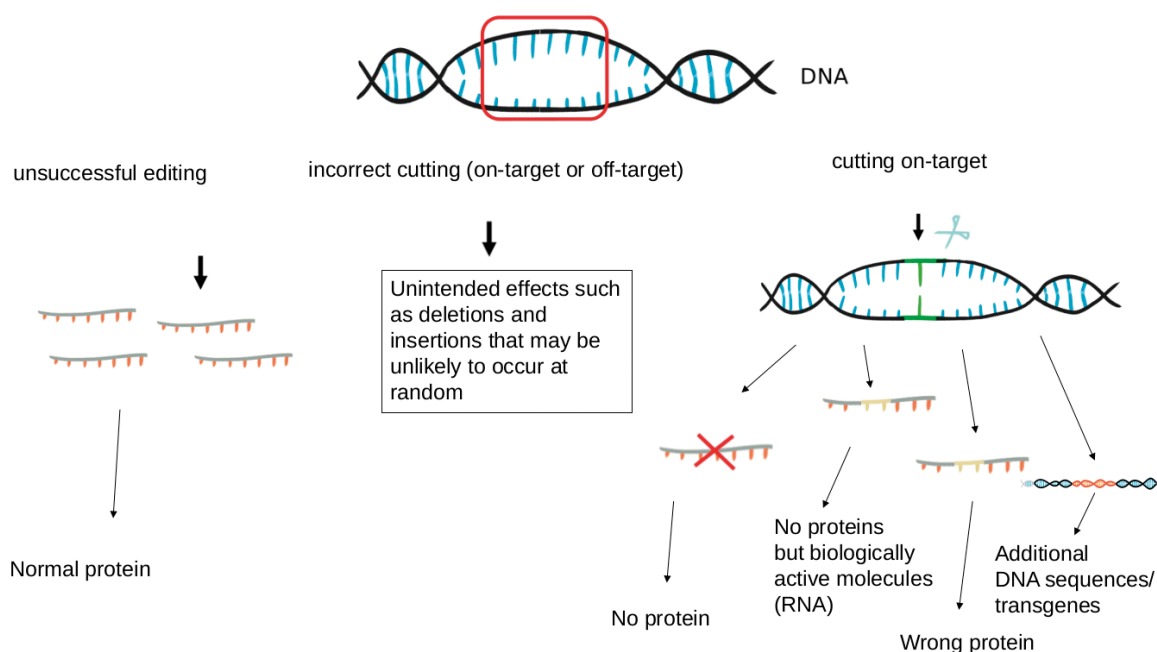


Figure 9: Overview of some types of unintended on-target and off-target changes caused by SDN-1 processes that can result in specific effects and create new depths in hazards and risk.

As already stated above, it is evident that each NGT application is linked to a specific potential for intended or unintended genetic alterations in the genome. As for regulated transgenic GMOs, safety now and in future can only be assessed in regard to specific 'events'. These 'events' are characterized by the techniques that are applied, the steps of the process and the resulting intended and unintended effects. It follows that, in regard to risk assessment, NGT-GMOs cannot be categorized according to the intended traits ('risk profiles') of the final organisms based on the intended characteristics of the final organisms (see Figure 10). Risk assessment also has to take into account the technical processes which were applied and the potentially resulting unintended effects (also see below).

If seeds with hazardous unintended genetic alterations remain undetected over longer periods of time, and are then used for further propagation, breeding and crossings, these genetic conditions may spread quickly and widely within the plant populations. Therefore, as required in EU regulation, in each case, intended and unintended changes have to be assessed as to whether they can have either direct or indirect, immediate or delayed, or cumulative effects on human health and on the environment.

5.2 Specific risks associated with NGT animals

The technical applications of NGTs in animals, at least in vertebrates, are associated with the risks and hazards of intended and unintended genetic changes. Similarly to plants, there are examples of NGT traits that are new or extreme variations of already known traits or new traits that are unlikely to result from random mutations and conventional breeding methods. Such traits can be associated with direct and indirect unintended effects that are relevant for risk assessment. In addition, there are also unintended genetic alterations in the target region (on-target effects) or in other genomic regions (off-target effects) that are specific to gene scissors, such as CRISPR/Cas, and are unlikely to occur from methods of conventional breeding.

5.2.1 Risks associated with intentionally introduced traits

It is obvious that traits such as those introduced into fish, e.g. seabream and pufferfish, can cause unintended effects that are triggered by the intended traits. As observed in GE pufferfish (Example 4.2.4), the blocked gene may be involved in metabolic functions. For example, the composition of the fish tissues can be altered and the susceptibility of the fish to diseases and infections may be increased. However, it is difficult to explore these questions since there are no peer reviewed publications or specific data. In addition to the risks, further questions need to be asked about health and animal welfare. In the case of the seabream, the animals have more muscle, they also have changes in body size and the vertebra are in an abnormal position (Kishimoto et al., 2018). Behavior also appears to have altered compared to the wild type since the NGT fish seem to move more slowly.

NGT pigs altered to increase their muscle mass are further examples of the many detrimental effects in regard to animal health. However, it cannot be finally concluded from the published data (Wang et al., 2015) whether these effects were caused by the intended traits or by unintended effects caused by the process, which also involved cloning at some stage. In this context it should be mentioned that the gene defect induced by NGTs in the seabream and the pigs, is also known to occur in the conventional breeding of cattle. However, the extreme effects observed in NGT-animals seem to be absent in conventionally bred species. One reason may be because the fitness of the animals is impacted. For example, in cattle, the conventional trait can only be established via additional technical measures, such as cesarean intervention at birth. It is astonishing that such a dubious trait is now being introduced with the help of NGTs in fish, pigs, sheep, goats and dogs (see, for example, Cohen 2019).

5.2.2 Unintended effects caused by the processes of New GE

In regard to unintended effects, there are many publications reporting potential medical applications (in animal cells or animals used in the laboratory). In this report, however, we can only include selected examples: experiments on human cell lines showed that cuts, also called double-strand breaks, caused by CRISPR/Cas gene scissors in the genome can lead to large, unwanted DNA rearrangements (see, for example, Geng et al., 2022; Leibowitz et al., 2021; Weisheit et al., 2020; Zuccaro et al., 2020) which may have detrimental effects during the early embryonic development of mammalian embryos (Papathanasiou et al., 2021).

In experiments with zebrafish, researchers have shown that unintended effects of CRISPR/Cas applications are inherited in subsequent generations (Höijer et al., 2022). The publication describes large structural changes at off-target sites. This shows that the gene scissors cut genomic regions outside of the target site, and thus cause specific unintended mutations. Many of the unintended genetic alterations have also been observed in the following generation. In some cases, the researchers found non-Mendelian patterns of inheritance, with some alterations being homozygous while others were heterozygous. The findings show that unintended effects caused by the gene scissors can lead to specific effects and risks.

Consequently, the offspring of animals manipulated with CRISPR/Cas for use in agriculture need to be examined in greater detail to detect unintended genetic alterations. This issue also seems to be relevant for genetically engineered hens: researchers in Israel used CRISPR/Cas to alter hens so that they do not produce male offspring (see Example 4.2.5). A deadly gene is passed on to any male offspring, which is meant to kill the male chicks (at early stage of development) before they hatch from the egg. As the research on zebrafish shows, surviving offspring may suffer from unintended genetic changes that can be associated with specific risks.

The most prominent example of unintended effects caused by NGTs are the hornless cattle in which the processes of genetic engineering caused genes from bacteria to be unintentionally integrated into the genome of the cattle (Example 4.2.2). In animal cells, it was found that unintentionally inserted foreign DNA fragments may not only come from the vector construct (Norris et al., 2020), but may also come from the genome of the bacteria used to multiply the vector DNA (e.g. *Escherichia coli*) or, surprisingly, taken up from the source of the growth medium, e.g. bovine or goat DNA, or retrotransposons (Ono et al., 2015, 2019).

Another study published in 2020 (Schuster et al., 2020) described the use of CRISPR/Cas to introduce the hornless trait in cattle. Being associated with many unintended effects, the publication shows just how complicated the processes of NGTs are: in this study, the scientists used CRISPR/Cas12a which is a variant of the “classic” CRISPR/Cas9 gene scissors. They took some skin cells for cloning from the ear of a Holstein-Friesian cow, a breed that is often used in milk production. They cultivated these cells in a cell culture and introduced the gene scissors into the cells together with a guide RNA to target the region in the cow genome coupled with a DNA template for the hornless trait (SDN-2). A total of 70 positive clones were produced in which the additional piece of DNA was inserted into the genome to convey the desired trait. The nuclei of the altered cells were then injected into previously denucleated (i.e. emptied of the nucleus) egg cells, which were then meant to develop into embryos. A total of nine embryos were transferred to surrogate cows. Three of the embryos did not induce pregnancy and died in the uterus. Four of the cows suffered serious complications in the course of their pregnancy and lost their calves. Another calf was killed prematurely for experimental purposes. Only one calf was born alive by caesarean section, but died the same day. It had malformations in several organs and also increased body weight. The causes of the serious damage to health were not examined in depth. It is likely that the cloning process played a major role in the undesirable outcome of the experiments, as cloning is known to result in birth defects. The study examined the genome of the genome-edited calf only to a limited extent in regard to unintended changes in the genome: PCR methods were used to search for off-target effects at three regions in the genome. Off-target effects are unwanted changes that can be caused by the gene scissors in parts of the genome that are very similar to the target sequence. No off-target effects were found in the three areas examined. However, the rest of the genome was not investigated. In addition, the scientists examined the genome by applying further PCR methods for unintentionally integrated DNA fragments. Their findings show just how limited the informative value of such a biased detection method is: the scientists could not completely rule out that there was additional antibiotic resistance in the calf genome. This was used for the work in the laboratory and should have no longer been present in the calf genome. In addition, the scientists could not clearly prove with the PCR method whether the integrated piece of DNA that mediates the hornless trait had been integrated into the calf genome once or several times. Only with a genome-wide analysis using whole genome sequencing methods would the scientists have been able to provide meaningful findings relating to the unintended changes.

5.3 Specific risks associated with NGT microorganisms

NGT microorganisms that are released may be able to survive and persist in the receiving environment, or invade new environments where they can have multiple interactions with other organisms. Even microorganisms not intended for release and whose purpose is for contained use only, may spread in the environment: experience with genetically engineered microorganisms used in food production processes shows that such applications may result in large-scale contamination with the bacteria or bacterial DNA (Deckers et al., 2021). Therefore, risk management questions relating to contained usage also have to be considered.

In general, many microorganisms are closely associated with species from other domains (plants or animals) that are considered to be their 'hosts'. The microbiome of plants, insects, mammals and humans are all made up of specific combinations of microorganisms. This means that the biological effects and potential adverse effects of NGT microorganisms may emerge from these symbiotic interactions in a non-linear pattern. These biological systems cannot, therefore, be assessed simply by examining their individual parts and pieces in isolation, they all have to be considered as a larger assemblages known as holobionts (or hologenomes when considering the total DNA of all involved organisms). It should also be taken into account that all species in the same habitat interact and influence each other (see, for example, Arif et al., 2020; Richardson, 2017; Sanchez-Canizares, 2017). It is not only the NGT microorganisms which may act upon target and non-target organisms, but also the host and the hologenome may impact the characteristics of the genetically engineered microbes. Furthermore, risk assessment of genetically engineered hosts, which may be combined with microorganisms by accident or on purpose, also needs to be considered.

These risks may have serious consequences for consumers. As EFSA (2022d) states in an opinion on what they consider to be SynBio microorganisms: *"Perturbation of the gut microbiome structure and microbial metabolism can also have consequences on the gastrointestinal (including metabolic, barrier defence and immune) function. Gut microbiome imbalances can impact epithelial integrity and, therefore, trigger adverse immune responses and inflammation. This can be of particular relevance in infants during the first months of life when severe disturbances of the gut microbiome balance and gut function may trigger chronic diseases at this point or later in life."*

5.4 Cumulative risks

Many organisms created with NGTs, across all kinds of species and different traits, may soon be released into the environment. Indirect, delayed and cumulative adverse effects arising from the releases may be more or less likely, depending on their specific biological characteristics (intended or unintended). Large scale releases may increase the likelihood of such effects.

Given the specific characteristics of NGT-GMOs as listed above, the legal requirement for assessing cumulative and long-term effects, which may have a wide-ranging impact on ecosystems, is a much more pressing issue in regard to GMOs derived from NGTs in comparison to previous applications of genetic engineering (see also Heinemann et al., 2021). There are at least two categories that need to be taken into account:

(1) Cumulative effects of NGT-GMOs belonging to several species:

environmental risk assessment that only takes single 'events' into account, may fail to predict or assess long-term cumulative effects, or possible interactions with the receiving environment and/or other NGT-GMOs. Consequently, although releasing low numbers of a single GMO derived from NGTs for a short period of time may possibly not result in adverse effects on the ecosystem, the combination with other NGT-GMOs or the release of larger numbers of a specific NGT-GMO over a longer period of time, might lead to a tipping point that would trigger irreversible damage. These cumulative effects may, for example, also be caused by interactions between NGT microorganisms and plants or animals, which raises challenges of potentially extreme complexity for risk assessment. For example, EFSA (2020b) in its draft opinion on the risk assessment of SynBio microorganisms (which also covers NGT-microorganisms) states: *"Even with the complete genetic information of a synthetic microorganism, it is beyond the capacity of any existent bioinformatic analysis to fully predict the capability of a synthetic organism to survive, colonise and interact with other organisms under natural conditions, given the uncountable diversity of potential microhabitats and their temporal variability."* (see also 5.6.)

(2) Cumulative effects from traits of NGT-GMOs within the same species:

NGT applications in one species or within a family of crossable species, which may also be susceptible to a specific range of pathogens, is a factor in potential cumulative effects, e.g. applications in wheat (*Triticum aestivum*) (see Example 4.1.6). The cumulative risk assessment in this case may face complex challenges. For example, cumulative effects of traits with (unintended) higher susceptibility to biotic stressors grown together with traits that have (unintended) reduced tolerance to abiotic stressors, may cause the collapse of plant populations which would otherwise have been successfully cultivated. Furthermore, different traits may be stacked via technical means, further breeding or also by spontaneous crossings, and thus result in offspring exhibiting biological characteristics absent in the parental plants. Under these circumstances, unintended genetic changes emerging from the processes of NGT may become relevant. This could magnify uncertainties and unknowns in regard to environmental risk assessment as well as the food and feed safety of NGT-GMOs.

In general, effects occurring from interactions that may be additive, antagonistic or synergistic, are hard to predict. Due to the intended and/or unintended effects emerging from different NGT traits established in one species, parallel cultivation, stacking or further crossing of the traits may cause unintended and even disruptive effects on plant health and response to biotic and abiotic stressors. These effects may be dependent on specific combinations of the traits and/or the exposure to stressful conditions. Even if each of the traits were to be considered 'safe', uncertainties or even unknowns will still emerge in the combination of the traits. Therefore, environmental risk assessment of the single traits may fail to predict or assess short- or long-term cumulative effects, or possible interactions with the receiving environment, or several traits in combination.

Similarly to environmental pollution with plastics and chemicals, it is not always an individual NGT-event which may create the real problems, but rather the sum of diverse effects on the environment. Environmental problems created by the release of NGT-GMOs may last as long as or longer than those caused by plastics and pesticides – thus impacting future generations.

5.5 A new dimension of hazards

In this context, hazards are defined as the potential of an organism to cause harm to or adverse effects on human health and/or the environment (EFSA, 2010; Commission Directive (EU) 2018/350). This report describes several NGT-GMO characteristics that may contribute to potential pathways causing such harm. The likelihood of damage occurring will also be dependent on exposure in the environment and the potential of the NGT-GMOs to persist, spread and propagate. Hazards include the disturbance or disruption of ecosystems as well as adverse health effects at the stage of consumption.

As aforementioned, the characteristics of the NGT-GMOs may contribute to potentially harmful pathways: for example, NGT camelina (Example 4.1.4) has a new genotype that is associated with a change in oil quantity and quality as well as other changes in plant metabolism. The food webs, the interaction with microorganisms and/or pollinators as well as natural defense mechanisms in the plants may all be disturbed (or even disrupted). Furthermore, any spread and propagation in the environment might lead to the offspring acquiring new characteristics absent in the original 'event' (see Bauer-Panskus et al., 2020). In addition, the degree of exposure in the environment will also be dependent on the potential of the NGT plants to persist, spread and propagate. In this case, the hazards include the disturbance or disruption of ecosystems (including detrimental effects on ecosystem-services involving beneficial and pollinating insects) as well as adverse health effects at the stage of consumption.

Overall, NGTs create a new dimension of hazards: the introduction of tools, such as CRISPR/Cas, enables a new depth of technical intervention at the level of the genome that, for example, can result in extreme variations in the traits as well as unintended genetic changes that are unlikely to occur with conventional breeding methods. Many of these effects are happening within a rapidly developing field with an increasing number of applications. Applications are not just confined to domesticated plants or animals, an increasing number of projects are investigating wild populations and a broad range of organisms, e.g. microorganisms, insects, rodents and trees, all of which are embedded in their own complex ecosystems.

There is growing evidence of complex interactions between plants and animals as well as genomic mechanisms that allow for resilience, adaption and co-evolution of ecosystems, populations and species. The underlying mechanisms of these evolutionary dynamics are scarcely understood. It has to be ensured that releases of NGT-GMOs do not negatively impact these natural dynamics within biodiversity by, for example, causing evolutionary mismatch effects between the NGT-GMOs and their environment, or by causing destabilization or disturbance of the natural networks of co-evolution and resilience.

It has also been shown that, for example, honeybees and pollinated plants can evolve together and survive conditions arising from climate change in what could be called an orchestrated process of development (Bartomeus et al., 2011). Genetically engineered organisms may promote evolutionary mismatch-effects within such complex interactions, and may thus interrupt the finely-tuned interactions between the species and the dynamics of co-evolution.

We also take into account that NGT-GMOs, such as honeybees, corals, amphibians, trees or crops, might look promising as short-term solutions. However, in the long-term, once these genotypes are introduced into complex natural networks and interactions, they may disturb and destabilize existing mechanisms of resilience and climate adaption. These considerations also underline the need for prospective technology assessment (see below).

Similarly to environmental pollution with plastics and chemicals, it is not always an individual NGT-GMO which may create the real problems, but rather the sum of diverse effects on the environment. The environmental problems created by the release of NGT-GMOs may not only be more diverse and complex but also last longer than those caused by plastics and pesticides – thus impacting many future generations.

This has created a potentially new dimension of hazards which could be triggered by potential releases of NGT-GMOs capable of rapidly overwhelming the adaptability of ecosystems. It is possible that NGT-GMO applications may, in addition to man-made effects such as climate change, contribute to the destabilization of ecosystems or intensify specific unfavorable effects. Given the high technical potential of the NGTs described above, assessment of the overall hazards linked to NGTs is potentially vital for averting the next man-made technology crisis and safeguarding planetary health (Horton & Lo, 2015). For this reason, there may be a case for generally restricting the introduction of organisms derived from genetic engineering into the environment.

5.6. Some requirements for the risk assessment of NGTs

As shown, each NGT application can be associated with the potential for specific intended or unintended genetic alterations of the genome. As pointed out, the risks not only depend on the trait, but also on the multistep processes of NGTs. These processes can be varied in many details, and thus adapted to the specific needs and purposes of each research team. Relevant details concern the way in which the nuclease is introduced into the cells, the types of nucleases used, the target regions, the species concerned and the experience of the researchers. Therefore, as established for regulated transgenic plants, the safety of the NGT-GMOs has to be assessed on a case-by-case basis and in regard to specific ‘events’ both now and in future. As explained above (see introduction), these ‘events’ are characterized by the techniques applied, the steps in the process and the resulting intended and unintended effects.

As far as their risk assessment is concerned, NGT-GMOs cannot be categorized e.g. simply according to their intended traits. However, as also discussed in the following section, it seems that the EU Commission is attached to the idea of categorizing NGT-GMOs in specific ‘risk profiles’, that might be based simply to their intended characteristics. The approach of the Commission was also taken up by EFSA (EFSA, 2022g) by proposing a decision making tree for risk assessment of NGT plants (‘edited plants’) which is solely based on the assessment of intended genetic alterations if no exogenous DNA sequence is present in the genome. If introduced into regulation, this would clearly lack sufficient basis in science. Many of the intended and unintended changes that can have either a direct or indirect, or immediate or delayed effects on human health and on the environment, would not have to undergo risk assessment as currently requested in Directive 2001/18/EC and the Commission Directive (EU) 2018/350. In particular, indirect, unintended, delayed and cumulative effects might be overlooked if these plans are put into regulatory practice. It would mean a fragmentation of current EU regulation in which only some selected ‘events’ still need undergo a mandatory approval process (see Figure 10).

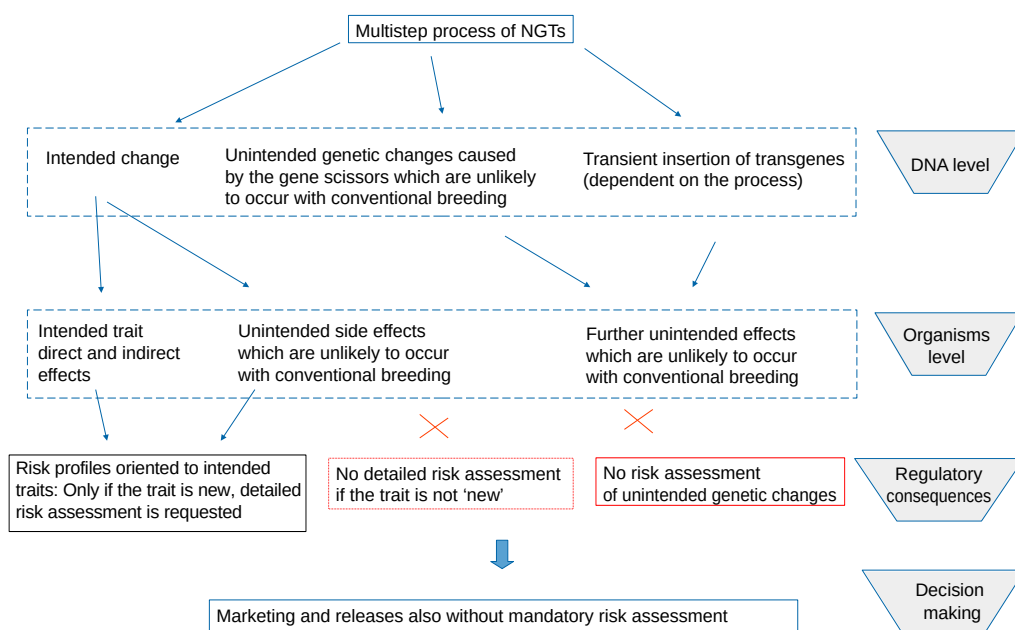


Figure 10: Scenario for a potential fragmentation of the EU GMO regulation for NGT plants derived from SDN (without the intended insertion of transgenes in the final organism) by introducing ‘risk profiles’ based on the intended traits

The unintended effects caused by the processes of ‘Old GE’ have also been identified and discussed in many EFSA opinions in relation to applications for the import of transgenic plants (www.testbiotech.org/en/database). There is no doubt that such effects must be assessed on a case-by-case basis to demonstrate the safety of the NGT plants. Whatever the case, alterations caused by the non-targeted insertion of transgenic elements in the first step of the process may remain in the plants and impact safety, even if the transgenic elements are removed by further breeding at the end of the process.

There is a general problem with the role of the European Food Safety Authority (EFSA) in this context. EFSA was requested by the EU Commission to investigate whether the current guidelines for the risk assessment of transgenic plants are sufficient for NGT plants. In their opinions, EFSA gives some attention to the intended traits (EFSA, 2020a, 2021, 2022b, 2022e). For example, as far as NGT tomatoes (Example 4.1.5) and NGT wheat (Example 4.1.6) are concerned, EFSA concedes that new methods, considerations and further developments in risk assessment approaches might be needed even if no additional genes are inserted in these events.

However, it looks like EFSA has not been asked to produce a report on the risks of NGT-GMOs in general, which would systematically take the unintended genetic changes and effects unrelated to the trait into account. As explicitly stated by EFSA (EFSA, 2020a and 2022f), their experts did not even conduct a full literature search to find a reasoned overview of unintended genetic changes, because this was not within their mandate. The number of publications waiting for the attention of EFSA has in the meantime increased substantially. Another sector which has so far been completely omitted from EFSA opinions, are effects emerging from interactions between NGT-GMOs that may be direct or indirect, immediate, delayed or cumulative.

It is likely that in several cases, larger uncertainties will remain, and therefore that make it hard to come to reliable conclusions on the safety of NGT-GMOs. Therefore, ‘cut-off’ criteria might be needed if decision-making is required in the face of greater unknowns (see Then et al., 2020). Against this backdrop, the EU Commission should require EFSA to explore and answer to the following questions and suggestions:

1. Which requirements are necessary and which methods are suitable for detecting and assessing specific unintended genetic and epigenetic alterations caused by the processes of NGT?

These alterations include unintended on-target and off-target effects, such as unintended insertions of transgenes, alterations of genomic regions which are especially protected by epigenetic factors, serial changes in off-target gene families or changes in regulatory elements. The aim would be to identify those genetic changes which are (alone or in combination) unlikely to occur in nature or result from conventional breeding (including random mutagenesis).

Relevant methods known are ‘Omics’ and include whole genome sequencing (WGS). According to a recent EFSA announcement (EFSA, 2022c), ‘Omics’ (which include genomics, transcriptomics, proteomics and metabolomics) may be routinely applied from 2030 onwards. However, these methods have so far not been requested.

2. Which requirements are necessary and which methods are suitable for detecting and assessing specific unintended effects caused by the NGT traits on the level of the organisms?

Such effects include, for example, unintended changes in gene expression which, besides food and feed safety, may seriously impact the development of changes in the composition of the organisms, changes in growth, susceptibility to biotic or abiotic stressors, circadian rhythms and time of flowering.

Relevant methods can be metabolomics (to identify and assess relevant changes in the metabolism of the organisms) and experimental exposure to a defined range of stress factors (e.g. such as those available in climate chambers or greenhouses) which, for example, can be used to investigate changes in plant fitness and response to climate change and pathogens. Such methods are available but have not so far been requested.

3. Which requirements are necessary and which methods are suitable for detecting and assessing direct and indirect, intended and unintended effects caused by the NGT-GMOs on the level of the ecosystems?

Such effects may, for example, include disturbances in the interactions with associated microbiomes, pollinators, food webs and specific non-target organisms. Underlying mechanisms may involve plant communication (exchange of information via specific molecules) with soil organisms, other plants, beneficial insects or pollinators. It also may include detrimental effects on species of wildlife feeding on NGT plants. These effects may also impact the stress resistance (resilience) of the ecosystem as a whole and its future evolutionary dynamics.

Relevant methods can be experiments in controlled environments such as artificial ecosystems (microcosm) and feeding studies. The definition of baselines and comprehensive knowledge about the ecosystems in the receiving environments is required. It is essential to identify uncertainties and limits of knowledge which may prevent the risk assessor from coming to final conclusions. Cut-off criteria might be needed since decision-making is required in the face of larger unknowns.

4. Which requirements are necessary and which methods are suitable for detecting and assessing specific intended and unintended effects caused by the processes of NGT with relevance for food & feed safety?

These may include, e.g. the uptake of biologically active substances in changed concentrations or with different biological effects compared to food products derived from conventional breeding. It is necessary to take cumulative aspects into account as well as indirect effects mediated via the intestinal microbiome. Relevant hazards include chronic adverse effects on health such as inflammation or metabolic disorders.

The findings from the risk assessment of the genome and the organisms are needed to assess the above issues. In general, whole food experiments should be used to assess the risks of relevant products at the stage of consumption. Also 'Omics', WGS and stress tests (at least for plants) should be used in each case to assess their impact on the safety of the food and feed products. Information on the intended traits, the applied methods, the intended and unintended changes, will help to define specific requirements and steps needed in risk assessment on a case-by-case basis.

5. Which requirements are necessary and which methods are suitable for detecting and assessing the cumulative effects of and interactions between different NGT-events?

Cumulative effects and interactions between different NGT traits may occur within one species (e.g. caused by stacking or parallel cultivation) or between NGT species which are released into a shared ecosystem. As set out above, it is not sufficient to perform risk assessment just for the single events.

Such effects may, for example, include disturbances in interactions with associated microbiomes, pollinators, food webs and specific non-target organisms. Underlying mechanisms may involve plant communication (exchange of information via specific molecules) with soil organisms, other plants, beneficial insects or pollinators. It may also include detrimental effects on wildlife species feeding on NGT plants. These effects may also impact the stress resistance (resilience) of the ecosystems as a whole and their future evolutionary dynamics.

In addition, if food or feed products from several events are mixed into a diet, cumulative effects must be investigated, and should typically examine both the whole food and feed mixtures.

Experience gained from currently grown transgenic plants shows that cumulative assessment is one of the major weaknesses in current regulation (Testbiotech, 2021f). Given the future expectation that many NGT traits will be released into the environment in short periods of time, these issues are becoming ever more pressing.

Relevant methods can include experiments in controlled environments, such as artificial ecosystems and feeding studies. This also requires the definition of baselines. However, comprehensive knowledge about the ecosystems in the receiving environments is not sufficient to assess interactions between NGT traits. In many cases, greater uncertainties and limited knowledge will prevent the risk assessor from coming to final conclusions. Therefore, cut-off criteria will be needed if decision-making is required in the face of greater unknowns.

6. Which requirements are necessary and which methods are suitable for establishing precautionary measures to prevent uncontrolled spread in the environment?

The persistence of NGT-GMOs in the environment in combination with spontaneous propagation and potential crossings with wild populations, may lead to biological characteristics being discovered in the offspring which were absent in the parental organisms (see Bauer-Panskus et al., 2020). This can be the cause of long-term effects which may disturb or even disrupt ecosystems and their future evolutionary dynamics. Existing experience shows that the risks and the hazards associated with the uncontrolled spread of regulated GMOs are generally underestimated, and next generation effects were in several cases just not predictable.

Relevant methods can include experiments in controlled environments to assess the fitness of the NGT-GMOs. In several cases, greater uncertainties and limits of knowledge will remain. Since long-term effects emerging from the uncontrolled spread of NGT-GMOs cannot be predicted, cut-off criteria are needed to prevent the release of NGT-GMOs into the environment if they have the potential to persist, spread and propagate.

6. Technology assessment (TA)

In the past, technologies that were supposed to solve problems (in the energy, food production and transport sectors) very often created new problems, e.g. climate change, nuclear waste, chemical pollution and extinction of species (see also EEA, 2001). A lack of awareness of the overall impact of releasing NGT-GMOs may have consequences for future generations. Therefore, in the context of NGTs, a technology assessment will be essential to address the potential benefits and possible drawbacks of NGT applications, including the overall impacts on ecosystems and socio-economic effects.

6.1 The role of a prospective technology assessment

Crucial principles for conducting technology assessment are, for example, summarized in the GAO “Technology Assessment Design Handbook” published in 2021: *“New technologies can have a range of effects, potentially both positive and disruptive, that TAs can explore. GAO has broadly defined TA as the thorough and balanced analysis of significant primary, secondary, indirect, and delayed interactions of a technological innovation with society, the environment, and the economy and the present and foreseen consequences and effects of those interactions.”* (GAO, 2021).

In general, case-specific risk assessment as foreseen in EU GMO regulation can be regarded as an ‘end-of-pipe’ safety control mechanism for individual organisms (events) just before they enter the market. In contrast, TA can deal more generally with (groups of) products derived from specific (new) technologies (ideally) before the final products reach the market.

6.2 Existing experience and the need for TA in the context of NGTs

Some stakeholders and political decision-makers are creating the impression that the hypothetical benefits of NGT plants are an actual fact. This impression, for example, can be seen in a text for an EU Commission consultation published in 2022.¹⁸ However, there is as yet no established regulatory framework to provide sufficiently clear and transparent standards or criteria needed to make evidence-based decisions on sustainability and potential benefits. There is a risk that the lack of sufficiently defined standards might lead to misinformation and market distortion.

In this context, experience gained from the first generation of transgenic crops should be taken into account; these were at the time not required to undergo TA. Despite many of the expected benefits never actually materializing, none of the products, such as herbicide resistant plants, were either sanctioned or removed from the market. The initial introduction of transgenic plants raised expectations of benefits for plant breeding and food security. Several publications claimed that the introduction of transgenic plants is associated with significant benefits (such as Brookes & Baarfoot, 2014; Gianessi & Carpenter, 2000; Kaphengst et al., 2010; Klümper & Qaim, 2014). However, other publications show that these expectations have either not or only partially been fulfilled. For example, the amount of herbicide used on transgenic crops was not reduced but increased, which means that the environment is particularly exposed to higher amounts of glyphosate (Benbrook, 2012; Schütte et al., 2017; Schulz et al., 2021). At the same time, the cultivation of plants producing insecticides suffers from pest replacement and resistance to the toxins (Cheke, 2018; Gassmann, 2021; Tabashnik et al., 2013; Xiao et al., 2021; Zhao et al., 2011). It appears that sufficiently defined criteria to assess the overall benefits of the introduction of transgenic plants are still missing.

¹⁸ https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/13119-Legislation-for-plants-produced-by-certain-new-genomic-techniques/public-consultation_en

Experience gained from transgenic plants can be used to exemplify the problem: for example, EFSA published an opinion in 2014 on the risk assessment of the transgenic soybean MON87769, which was engineered to have a positive effect on health by increasing the concentration of Omega-3 fatty acids in food products (EFSA, 2014). EFSA, however, did not assess the claims made by Monsanto about the benefits to health, and was not able to assess any long-term effects from the consumption of these food products. Nevertheless, the Commission authorized the import of the soybeans for food production. It is very likely that now there will be many more applications filed for the approval of products derived from NGT plants with claimed health effects which not only have to be checked for risks, but also checked for claimed benefits which are outside of the mandate for EFSA risk assessment.

There is some interlinkage with sectors outside the GMO regulation: for example, EU Regulation 1924/2006 requests the assessment of potential benefits from food health claims. However, these requests are only directed to specific products in isolation, while combinatorial effects are not assessed. In light of NGT products, such as tomatoes with enhanced content in GABA (see Example 4.1.3), Vitamin D¹⁹ and other changes in fruit composition (see Example 4.1.5), it is evident that not only the single event, but also its overall (positive or negative) health effects need to be considered if the fruits are mixed together.

There are further problems in context of the ‘Green Deal’ and the claims made in regard to the sustainability of NGT plants or animals used in agriculture (see below). In order to achieve the goals of the ‘Green Deal’ and implement the ‘Farm to Fork’ strategy, clear and reliable criteria is a prerequisite for making decisions on the hypothetical benefits of each ‘event’ and the technology as a whole. In worst case, insufficiently regulated NGTs may block systemic options needed to achieve more sustainability.

NGTs cannot be regarded as a transformation technology in the same way as renewable energy which is necessary to shut down energy production from fossil fuels. There is no basis for proposing that NGTs should widely replace traditional breeding. Currently, it is not possible to predict the extent, the purpose, in which circumstances or for what outcomes NGT could be applied in plant production. There are some interesting examples of proof of concept and a lot of (often questionable) promises, but no criteria on how to identify real needs, ‘true’ benefits or potentially disruptive effects on the economy and/or ecology.

In this context, it is important to emphasize that the risk assessment (of the single events) and TA (the systemic effects of food production systems) are organized in separate regulations and should not be confused with each other. The requirement to demonstrate safety of the NGT-GMOs and products derived thereof should in no way be compromised because of expected benefits.

Therefore, additional regulatory framework for TA should be established before NGTs enter the EU market. This framework should be appropriate for considering potentially beneficial effects as well as other impacts of the technology not related to the safety of specific ‘events’. The justification for this additional framework lies in the unique complexity and the potential irreversible consequences of the large-scale introduction of NGTs into the environment, agriculture and food production.

Consequently, in addition to a mandatory case-by-case risk assessment, the priority for political decision-makers should be a complementary regulatory framework for prospective TA. It should include robust criteria to assess potential benefits of NGTs for production systems and the environment. In this way, TA would represent a second level of scrutiny (additional to case-specific risk assessment) to evaluate whether these technologies are really needed and suitable for solving the problems at hand (see Figure 2).

19 <https://www.testbiotech.org/en/news/crispr-tomatoes-created-produce-vitamin-d>

In particular, there is a requirement for clear, transparent, reliable and enforceable assessments, standards and criteria, which allow evidence-based decisions to be made on sustainability and potential benefits predicated on the basis of a comprehensive TA. The criteria should take into account alternatives which are based on conventional breeding, agroecology or other sectors within the food production systems. In future, to avoid unnecessary risks the regulator should aim to prevent releases of any NGT plants based on non-justified claims and empty promises.

6.3 TA & NGT impacts on food production

It can be assumed that, if there is a large-scale introduction of NGTs into agriculture, this will not only affect the characteristics of distinct crops and livestock, but will also have extensive impacts on food production systems as a whole.

NGTs can be considered to be disruptive technologies which will impact ecosystems as well as social and economic systems of food production if introduced into agriculture on a large-scale. Ledford (2015) characterizes CRISPR/Cas as a 'disruptor' in several senses: a powerful technology and a big game-changer with huge potential, but not without serious concerns. Experts, such as Goold et al. (2018) and Menchaca et al. (2020), praise the (beneficial) disruptive potential of CRISPR/Cas technology, especially in the field of agriculture, fisheries and forestry. On the other hand, there are warnings of potentially (negative) disruptive effects on seed markets, food production and consumer choice (Clapp 2021; Testbiotech 2021a). Impacts on the concentration of seed markets have already been observed in connection with transgenic plants (see, for example, Bonny S., 2017; Clapp, 2021; Howard, 2009; Howard 2015).

Considering NGTs as disruptive technologies does not imply that they are per se are good or bad. It simply means that disruptive effects will occur on several levels with effects that deserve prospective assessment. For example, disruptive effects may center on the use of pesticides and fertilizers, which may be significantly reduced or increased by the introduction of NGT-GMOs.

NGTs may also become disruptive in another sense if coexistence, labeling and traceability are weakened or fragmented. Under these circumstances, consumer choice, organic agriculture and non-GE food production may be severely hampered or disabled. Food security, food sovereignty and freedom of choice for farmers and consumers should, therefore, be taken into account in prospective TA.

In this context, the accessibility of proprietary technology as well as access to biological material needed for all plant innovation has to be considered. Patents play a particularly crucial role in this context since they can be used to block, hamper or control access to technology and biological resources (Tippe et al., 2022).

In general, TA should also take socio-economic factors into account, e.g. to assess a potential redistribution of costs and profits and/or of benefits and disadvantages within the food production systems. More specifically, the impacts on traditional breeding and food production, organic agriculture and consumer choice have to be taken into consideration, as also concluded by the EU Commission (EU Commission, 2021). As mentioned, monopolistic control of biological resources needed for plant and animal breeding through patents is another issue which will need in-depth analysis (Testbiotech, 2021a).

More generally, if traditional food production systems are to be replaced or combined with new disruptive technologies, then the wider consequences should be assessed beforehand. NGT applications are likely to affect close-knit networks, including small and medium sized breeders, traditional farmers and regional markets typical of European food production systems. In this context, fundamental questions need to be asked such

as what consequences a disruptive technology may have in the context of food production embedded in a network of seed diversity, biodiversity, natural resources and ecosystems.

6.4 TA and NGT impacts on sustainability

TA is especially relevant when it comes to the expected benefits of releasing NGT-GMOs, such as mitigating the impacts of climate change or promoting sustainability in agriculture and food production. Guidances and criteria may be needed to distinguish traits with ‘real benefits’ from those which are simply ‘empty promises’. As mentioned, the introduction of transgenic plants thirty years ago came with high expectations in regard to the reduction of pesticides and beneficial health effects (see for example, OECD, 1992). However, many of these expectations failed to materialize. How can we avoid similar developments in the context of NGT-GMOs?

Without comprehensive TA based on clearly defined criteria, any claims made about the sustainability of NGT applications are largely the result of wishful thinking and speculation. It is important to be aware that there is so far a lack of data and reliable findings. In addition, no conclusions can be drawn from potential applications as listed, for example, by JRC (2021). At the moment, there are many technical options but no proof of any beneficial effects. On the other hand, if released without sufficient control, NGT-GMOs might destabilize the ecosystems and may even become a new driver of species extinction. The relevant questions for TA in this context are, for example, whether tipping points can be predicted which may cause ecosystems to collapse by overwhelming them with NGT-GMOs.

While TA cannot replace the risk assessment of the individual organisms (‘events’), it is nevertheless necessary in political decision-making to seek a balance between the potential benefits and reducing the overall risks of adverse effects on biodiversity and planetary health. If NGT-GMOs are, for example, introduced into agriculture, their potential negative impacts may be minimized by only approving plants or animals which are considered to be safe, and where there is a reasonable expectation of them providing substantial benefits.

6.5 Some alternatives to NGTs

TA should also take into account alternatives based on conventional breeding, agroecology or other experiences within the food production systems. Table 2 is derived from international patent applications published in 2020 (Tippe et al., 2021) which in many cases claim the application of NGTs, even though the examples included in the patents actually describe conventional breeding methods (including random mutagenesis). Therefore, these patents show that many of the benefits claimed for NGTs are also achievable using conventional methods. This also shows that if the patents are granted as filed, the scope of the patent would cover both NGT-GMOs and conventionally bred plants. Patents on NGTs may, therefore, become disruptive to conventional breeding by hampering or blocking access to biological diversity needed by all breeders.

Table 2: Examples of traits that are achievable using conventional breeding and that are of relevance to sustainable agriculture and/or climate change mitigation by increasing tolerance to biotic and abiotic stressors, taken from international patent applications from 2020 (Source: Tippe et al., 2021)

Patent number	Species / Trait
WO2020074237	Lettuce / downy mildew resistance
WO2020168166	<i>Brassica</i> plants / clubroot resistant
WO2020239496	<i>Cucurbitaceae</i> / downy mildew resistance
WO2020239495	Tomato and cucumber / oomycete resistance
WO2020132188	Maize (sweet corn) / resistance to Northern leaf blight
WO2020036950	Oilseed rape / blackleg resistance
WO2020036954	Oilseed rape / blackleg resistance
WO2020139756	Cotton / resistance to fusarium
WO2020051166	Several species / tolerance under water stressed conditions
WO2020229533	Maize / drought tolerance in maize
WO2020025631	<i>Cucurbitaceae</i> / resistance to Cucurbit Yellow Stunting Disorder virus (CYSDV)
WO2020128044	Spinach / resistance to <i>Peronospora farinosa</i>
WO2020193712	<i>Brassica</i> plants / resistance to downy mildew
WO2020035145	Lettuce / resistance to downy mildew
WO2020126500	Lettuce / resistance to downy mildew
WO2020148021	Tomato / resistance to Tobamo virus
WO2020239186	Melons / resistance to downy mildew
WO2020099330	Potato / resistance to <i>Phytophthora infestans</i>
WO2020239215	Spinach / resistance to downy mildew
WO2020077224	Pepper plants / resistance against fusarium
WO2020030804	Cauliflower / resistance to <i>Xanthomonas campestris</i>
WO2020249798	Tomato / resistance to Tobamo Virus
WO2020234426	Rice / increased yield
WO2020248971	Maize / changed architecture
WO2020260890	Wheat / increased water efficiency

TA should also take into account alternatives which are based on traditional breeding. Since plants and animals derived from conventional breeding (including random mutagenesis) can be expected to typically be associated with less uncertainty regarding hazards and risks, they should be given priority.

Furthermore, as science in agroecology shows, the overall system of agriculture and the choice of crops to be grown is likely to have far more impact compared to breeding of individual varieties in the context of climate change and sustainability (see, for example, Poux & Aubert, 2018). Genetic diversity within species and ecological networks is key to providing a sufficiently broad range of possible solutions. Against this backdrop, there is an abundance of scientific evidence in support of strategies aiming to increase diversity in agroecology systems (see FAO, 2017). The same is true for forests (see, for example, Morin et al., 2018) and grasslands (see, for example, Isbell et al., 2015).

7. Requirements for NGT regulation and decision-making against the backdrop of the precautionary principle

As shown, political decision-making on the future regulation of NGT-GMOs is likely to be faced with huge challenges. There is, furthermore, evidence that the intrinsic factors of NGTs deserve more attention from the regulators. For example, according to Yang et al. (2022), “*mutation locations and scales, potential off-target modifications, complexity of the introduced changes, and novelty of the developed traits*” make it necessary to apply “*rigorous research on genome-editing applications and reliable techniques for risk assessments of genome-edited plants*”.

Kawall (2021a), in investigating the generic risks associated with the application of the CRISPR/Cas machinery, concludes, “*In summary, this review here shows that about half of the market-oriented plants developed by SDN-1 applications contain complex alterations in their genome (i.e., altering multiple gene variants or using multiplexing). It also illustrates that data on both the process- and the end-product are needed for a case-by-case risk assessment of genome edited plants. The broad range of genetic alterations and their corresponding traits reflects how diverse and complex the requirements are for such a risk assessment.*”

Eckerstorfer et al. (2021) come to a similar conclusion: “*To this end, we suggest that two sets of considerations are considered: (1) trait related-considerations to assess the effects associated with the newly developed trait(s); and (2) method-related considerations to assess unintended changes associated with the intended trait(s) or with other modifications in the GE plant (...) Based on these considerations, further guidance should be developed to ensure the high safety standards provided by the current regulatory framework for GMOs in the EU for GE plants in an adequate and efficient way, taking into account the existing knowledge and experience in a case-specific manner. This guidance should thus strengthen the case-specific approach that is recommended by numerous EU and Member States institutions.*”

Consequently, all NGT-GMOs need to undergo a mandatory approval process before being released into the environment or brought to market. Furthermore, a comprehensive and prospective TA is necessary to address systemic risks to biodiversity, socio-economic impacts and effects in regard to sustainability (see Figure 2) before NGT-GMOs are used in agriculture. In accordance with the precautionary principle, a technology assessment should also include an in-depth consideration of the need for the technology and consider the alternatives that could be made available. One aim should be to generally restrict the number and scale of releases of NGT-GMOs into the environment in order not to lose control in regard to potential cumulative adverse effects on health and the environment, and also to avoid passing potential tipping points for irreversible damage to ecosystems.

The overarching question which this report seeks to answer is: “*What are the crucial requirements for an ideal regulation of NGTs from the perspective of the protection goals regarding health, the environment and animal welfare?*”

The report presents the following conclusions and answers:

EU regulation of NGTs should prevent:

- uncontrolled marketing or releases of genetically engineered (NGT) organisms into the environment;
- damage to biological diversity, ecosystems and agriculture;
- health hazards from being introduced unnoticed into the food system where they might accumulate;
- data needed for risk assessment by independent experts being treated as confidential business information;
- contamination of organic and other food or seed production systems which exclude the use of genetically engineered organisms.

EU regulation of NGTs must ensure:

- a case-by-case risk assessment and an approval process for each NGT event, including taking accumulated effects into account;
- the further development of data requirements, guidelines and methods of risk assessment to achieve the highest safety standards, including cut-off criteria in cases where uncertainty is too great;
- the availability of information to track and trace the NGT-GMOs and food products derived thereof;
- measures are in place to prevent the uncontrolled spread of NGT-GMOs in the environment;
- consumer choice and coexistence with organic and GE free food production;
- animal welfare is fully respected at all stages of the NGT processes;
- a prospective and comprehensive TA is carried out before NGTs are brought to market.

In summary, EU regulation should uphold the precautionary principle and give priority to safety for human health, the environment and nature. Current EU GMO regulation is an adequate framework but will need some adjustments in regard to its implementation by EFSA. It should, in addition, be augmented by prospective TA (Figure 2). As it stands, the current regulatory framework should not be fragmented by introducing lower standards for some groups of NGTs (Figure 10).

8. Answers to questions in the TOR

a) Are there any reasons not to exempt some specific groups of NGTs from EU GMO regulation?

Current GMO regulation requests mandatory approval procedures for each ‘event’ (‘case-by-case’) if they are derived from processes of genetic engineering, including NGTs. The regulations require that safety is demonstrated before approval is issued. The applicant is required to supply the necessary data. The regulation further requires the inclusion of detection and traceability methods. After market approval is issued, the regulations also require post-market monitoring.

This regulatory framework appears to be adequate. As shown in this report and also concluded by Eckerstorfer et al. (2021), there is no scientifically justified way of delineating specific categories of NGTs which can be regarded as being safe without in-depth risk assessment. Only after the necessary data are provided and assessed independently, can a conclusion on safety be drawn. In addition, if some groups of NGTs are exempt from current EU GMO regulation, this would weaken detection, tracking and monitoring.

b) What is the current state of knowledge about identification of NGT and what are the implications for risk assessment, approval process, traceability and labeling of NGT?

The application of site-directed nucleases (SDNs), such as CRISPR/Cas, will in most cases lead to typical patterns of genetic change and these patterns can be used for identification and traceability. For example, unlike random mutagenesis, NGTs are able to knock out genes which are present as multiple copies in the plants. Thus, whenever a crop is found in which multiple copies of the same gene have been knocked out, it will almost certainly be an NGT product. Consequently, plants changed by NGTs can usually be very clearly distinguished from other plants. For most NGT products, a clear signature can be found in the DNA, for

instance, where the exact same nucleotide stretch is erased. If that signature is revealed by the developer, then PCR technology can in most cases be used to detect and monitor genome-edited products. The typical patterns of genetic change as well as specific alterations of single DNA sequences will allow the identification and traceability of NGT-GMOs in most cases (Duensing et al., 2018). In cases where the traits derived from NGT are similar to those derived from conventional breeding, the unintended changes caused by the processes of NGTs can often be identified, and thus used to track and trace the relevant organisms.

Therefore, it is essential to ensure that the companies provide the necessary data during the mandatory approval process. Typically, methods of detection and identification will be possible if the relevant data are provided. In addition to PCR, whole genome sequencing, metabolomics and information from international registers as well as documentation transmitted through the operator chain, may all be combined to detect and identify the NGT plants (BfN, 2022).

Consequently, if whole groups of NGTs were to be exempt from mandatory approval processes, this is likely to have negative impacts on the availability of detection methods, traceability, coexistence, consumer choice and post-market monitoring.

c) What is the state of knowledge about unintended effects and how should they be taken into account within risk assessment?

In several cases, unintended genetic alterations in the target region (on-target) or in other genomic regions (off-target) that are specific to gene scissors, such as CRISPR/Cas, have been described in plants (see, for example, Biswas et al., 2020; Braatz et al., 2017; Eckerstorfer et al., 2021; Hahn & Nekrasov, 2019; Kawall, 2021a; Yang et al., 2022). For example, larger structural genomic changes, such as translocations, deletions, duplications, inversions and scrambling of chromosomal sequences, can occur near the SDN target site (as well as at the SDN target site) which otherwise would be unlikely to occur (see e.g., Hahn & Nekrasov 2019). In addition, specific unintended on-target effects often include the integration of DNA from vector DNA derived from transformation processes, where, for example, bacterial DNA was unexpectedly integrated (e.g. Andersson et al., 2017; Li et al., 2015; Zhang et al., 2018). Overall, the CRISPR/Cas9 system has been confirmed to have a high frequency of integration at the target site, and hence in large deletions (Lee et al., 2019; Yang et al., 2022). As a result, similarly to cases where there are intended effects, unintended effects can also be caused by patterns of unintended genetic changes that go beyond what can be achieved with conventional breeding, and thus result in specific risks.

In addition, it has to be taken into account that New GE is a multi-step process, with inherent and specific risks that occur independently of the desired traits. As mentioned above, in plants, NGTs such as CRISPR/Cas typically make use of older genetic engineering ('Old GE'), e.g. non-targeted methods such as *Agrobacterium* transformation or biolistic methods ('gene canon'), to deliver the DNA for the nuclease into the cells. As shown, the mechanisms and outcomes of these abovementioned technical processes for the insertion of genes cannot be generally equated to effects occurring naturally or with previously used breeding methods. In conclusion, the processes used for the technical insertion of DNA can cause effects which are different in their scale, their sites, patterns of genetic change and their biological characteristics when compared to those of non-regulated breeding methods or natural processes.

In summary, at each stage of the process – including (i) insertion of the DNA of the gene scissors into the cells, (ii) target gene recognition and cutting, and (iii) cellular repair of the genes – specific unintended alterations can occur that are associated with risks (for an overview, see Kawall et al., 2020).

d) What is the state of knowledge about the specific risks of NGTs?

This report shows that the technical potential of NGTs and their risks and hazards are closely interrelated. We include selected applications to exemplify specific hazards and risks. In summary:

(1) Many NGT traits are unlikely to be achievable with random mutation and conventional breeding methods, even if no new gene functions are inserted (see also Examples 4.1.3-4.1.6). These traits did not evolve from evolutionary processes and may have unintended direct or indirect effects on the environment, and may also put food safety at risk (Kawall, 2021a).

(2) Unintended genetic changes have been observed (on-target and off-target) that are specific to the multistep processes of NGTs and unlikely to occur due to random mutation or conventional breeding processes. These genetic irregularities are associated with risks inherent to the technical processes (Kawall et al., 2020; Kawall, 2021b).

Both the unintended effects caused by the intended traits and those caused by unintended genetic alterations may result in a new dimension of hazards. This is especially relevant when it comes to cumulative effects, which may be additive, antagonistic or synergistic. Even if the single traits were deemed 'safe', uncertainties or even unknowns will still emerge in the combination of the traits. Therefore, environmental risk assessment of the single traits may fail to predict and assess long-term cumulative effects or possible interactions with the receiving environment, including combinations of several traits.

Similarly to the spread of non-native diseases that are frequently vectored by non-native species or human activities, genetically engineered organisms introduced into natural populations may severely impact animal, plant and human health as well as damaging biodiversity and planetary health. The potential releases of NGT-GMOs represent a new dimension of hazards that may overwhelm the adaptability of ecosystems. Thereby, NGT-GMOs applications may, in addition to man-made effects such as climate change, contribute to a destabilization of ecosystems or intensify specific unfavorable effects. Therefore, in addition to risk assessment, prospective TA should also be required in order to avoid passing potential tipping points which could cause irreversible damage to ecosystems.

e) What are the reasons for case-by-case risk assessment (approval process for each event) and under which conditions (burden of proof, evidence of safety) a whole group of NGTs could be handled in the same way?

As shown, the processes used to generate NGT plants are generally complex and have both intended and unintended effects. Many of these effects are unlikely to emerge from methods of conventional breeding (including random mutagenesis) (see, for example, Kawall, 2019; Kawall, 2021 a/b, Eckerstorfer, et al., 2021). Therefore, the idea that introducing 'risk profiles' as proposed by the EU Commission²⁰, may allow any conclusions to be drawn on the safety of NGT plants without detailed risk assessment cannot be justified from a scientific point of view. Rather, all organisms derived from NGT processes should also in the future undergo a mandatory approval process and detailed risk assessment, taking the precautionary principle into account.

However, it should be acknowledged that the existing regulatory framework already provides the flexibility needed to adapt the guidelines to the risk assessment of various NGT applications. Further development of methodologies currently applied in risk assessment is not something that should cause major problems. Such adaptations may allow the categorization of the requirements for risk assessment of certain groups of NGTs, for example, taking into account the techniques used to introduce the nucleases into the cells. However, even within these categories, each 'event' ('case-by-case') needs to undergo individual risk assessment.

20 https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/13119-Legislation-for-plants-produced-by-certain-new-genomic-techniques_en

f) Which methods are available to not only assess the intended traits of a plant but also its unintended biological characteristics?

On the level of the genome, the aim would be to identify those genetic changes which are (alone or in combination) unlikely to occur in nature or as a result of conventional breeding (including random mutagenesis). Relevant methods are known as ‘Omics’ and whole genome sequencing (WGS). According to a recent announcement by EFSA (EFSA, 2022c), ‘Omics’ (which include genomics, transcriptomics, proteomics and metabolomics) may be routinely applied from 2030 onwards. However, they have so far not been requested. Risk assessment of NGT-GMOs should not be carried out without these methods being integrated.

On the level of the organisms, ‘Omics’ (Metabolomics) should also be applied. The aim here would be to identify changes in gene expression and composition. For this purpose, experimental exposure to a defined range of stress factors (such as those in climate chambers) should be used to investigate changes in plant fitness and response to climate change and pathogens. Such methods are available, but have so far not been requested. Again, risk assessment of NGT-GMOs should not be carried out before these methods are integrated.

On the level of the ecosystems, detailed risk assessment will be needed before the plants are introduced into the environment. Suitable methods include experiments in controlled environments, such as artificial ecosystems and food webs. The definition of baselines and comprehensive knowledge about the ecosystems in the receiving environments is required. It is essential to identify uncertainties and limits of knowledge which may prevent the risk assessor from coming to final conclusions. Cut-off criteria might be needed if decision-making is necessary in the face of greater unknowns, such as those caused by cumulative risks, and also to prevent uncontrolled spread.

In regard to food and feed safety, whole food experiments should be used to assess the risks of relevant products at the stage of consumption. The findings from the risk assessment on the level of the genome and the organisms have to be taken into account. Information about the intended traits, the applied methods, the intended and unintended changes will help to define the further steps in risk assessment. Typically ‘Omics’, WGS and (for plants) stress tests should be applied in each case. Furthermore, if products from several ‘events’ are mixed into a diet, potential cumulative adverse effects have to be investigated in whole food or feed mixtures. In addition, and independently of risk assessment, the regulatory framework has to ensure that potential beneficial health effects are assessed if intended by the specific traits (alone and in combination with other traits).

g) What is the state of knowledge about the differences in genetic changes caused by NGTs compared to the natural mutations?

If compared to natural mutations and methods of conventional breeding (including random mutagenesis), NGTs can overcome the boundaries of natural genome organization which has emerged from evolutionary processes. Relevant factors include repair mechanisms, gene duplications, genetic linkages and further epigenetic mechanisms (see, e.g. Belfield et al., 2018; Filler Hayout et al., 2017; Frigola et al., 2017; Halstead et al., 2020; Huang & Li, 2018; Jones et al., 2017; Lin et al., 2014; Monroe et al., 2022; Wendel et al., 2016), and thus make the genome much more extensively available for genetic change (Kawall, 2019; Kawall et al., 2020). The resulting genotypes (the patterns of genetic change) can be vastly different compared to those derived from conventional breeding, both in regard to intended and unintended changes (Kawall, 2021a/b). This means that it is possible to generate genotypes which are highly unlikely to result from natural processes or traditional breeding techniques. Even without the insertion of additional genes, changes in genotypes and phenotypes can be pervasive and brought about by, for example, knocking out very many or all copies of a gene family, thus

changing several genes in parallel (multiplexing) or altering elements responsible for gene regulation (Kawall et al., 2020; Raitskin and Patron, 2016; Wang et al., 2016; Zetsche et al., 2017). Such technical interventions can lead to major and unprecedented changes in plant composition, which may also be associated with unintended effects and specific risks (EFSA, 2022b; Kawall, 2021a/b; Nonaka et al., 2017; Sanchez-Leon et al., 2018). In conclusion, natural mutations and genetic changes caused by NGTs may be compared with each other, but cannot be equated.

h) Are claims justified that NGTs are absolutely necessary to generate plants which are adapted to climate change?

Evolution has given many species deep links to earlier stages of evolution with similar environmental conditions (see, for example, Shubin, 2020). Recent research has found that, in many cases, it is not a new mutation or new genetic conditions that are needed for survival, but solutions to problems – and these may already be available in the genome of the species or in the networks of ecosystems such as corals (summarized in Testbiotech, 2021b). Therefore, it is not surprising that many of the desired traits as mentioned in context of NGTs are indeed achievable using conventional breeding, which makes use of biodiversity within the boundaries and potentials that have emerged from evolution.

More generally, evolution builds on genetic and biological diversity which, as a system, can continue to evolve, very often using already existing solutions to problems. It is not simply about the single ‘fittest’ organism to survive, but about populations and ecosystems which are diverse and flexible enough to respond to new environmental conditions. As a result, genetic diversity within species and ecological networks is key to providing a sufficiently broad range of possible solutions. Against this backdrop, there is an abundance of scientific evidence in support of strategies aiming to increase diversity in agroecological systems²¹. The same is true for forests (see, for example, Morin et al., 2018) and grasslands (see, for example, Isbell et al., 2015).

On the other hand, there is as yet no established regulatory system for NGTs to provide sufficiently clear and transparent standards or criteria needed to make evidence-based decisions on sustainability and potential benefits. Consequently, in addition to the mandatory case-by-case risk assessment, political decision-making should aim to establish a corresponding regulatory framework for prospective TA. It should include robust criteria to assess the potential benefits of NGTs for production systems and the environment. As such, TA would represent a second level of scrutiny (additional to the case-specific risk assessment) to evaluate whether these technologies are really needed and suitable to solve the problems at hand.

While TA cannot replace the risk assessment of the individual organisms (‘events’), it is nevertheless necessary in political decision-making to seek a balance between the potential benefits and reducing the overall risk of adverse effects on biodiversity and planetary health. If NGT-GMOs are, for example, introduced into agriculture, their potential negative impacts may be minimized by only approving plants or animals which are considered to be safe, and where there is a reasonable expectation of them providing substantial benefits. Therefore, the EU should establish a framework which allows the prevention of any releases of NGT plants based on non-justified claims and empty promises.

²¹ <http://www.fao.org/3/I9037EN/i9037en.pdf>

i) What are the pros and cons of NGT processes in animal breeding?

The examples presented above highlight several dilemmas: on the one hand, the traits introduced by NGTs, such as hornless cattle (4.2.2) and cattle with ‘slick’ coats (4.2.1) are claimed to be beneficial to animal welfare. However, these traits are also achievable with conventional breeding whereas, in comparison, NGT processes caused additional unintended changes in the genome. Therefore, from the perspective of TA, these applications may appear neither necessary nor useful.

Other traits also seem to follow questionable goals – such as in the case of fish that gain weight faster or fish and pigs with higher muscle mass (4.2.3 and 4.2.4.). Finally, there are traits such as Example 4.2.5 (hens with no male offspring) or those listed in the Euginius database, e.g. pigs with disease resistance (GE-CD163 Pig) or with allergen reduction (GalSafe pig), which cannot be discussed in more detail since the relevant data appear to be missing.

In general, NGT methods, at least if applied in vertebrates, are not neutral in regard to animal welfare. Methods of cloning, the involvement of surrogate mothers and also the intended traits are often associated with animal suffering (see, for example, Schuster et al., 2020). Therefore, prospective TA should be applied to avoid NGT applications in vertebrates which are not absolutely necessary. This requirement is also in accordance with EU animal welfare legislation.

j) In summary: What are the crucial requirements for an ideal regulation of NGTs from the perspective of the protection goals regarding health, the environment and animal welfare?

EU regulation of NGTs should prevent:

- uncontrolled marketing or releases of genetically engineered (NGT) organisms into the environment;
- damage to biological diversity, ecosystems and agriculture;
- health hazards from being introduced unnoticed into the food system where they might accumulate;
- data needed for risk assessment by independent experts being treated as confidential business information;
- contamination of organic and other food or seed production systems which exclude the use of genetically engineered organisms.

EU regulation of NGTs must ensure:

- a case-by-case risk assessment and an approval process for each NGT event, including taking accumulated effects into account;
- the further development of data requirements, guidelines and methods of risk assessment to achieve the highest safety standards, including cut-off criteria in cases where uncertainty is too great;
- the availability of information to track and trace the NGT-GMOs and food products derived thereof;
- measures are in place to prevent the uncontrolled spread of NGT-GMOs in the environment;
- consumer choice and coexistence with organic and GE free food production;
- animal welfare is fully respected at all stages of the NGT processes;
- a prospective and comprehensive TA is carried out before NGTs are brought to market.

In summary, EU regulation should prioritize the precautionary principle as well as safety for health, the environment and nature. Current EU GMO regulation is an adequate framework, but will need some adjustments in regard to its implementation by EFSA and should also be augmented by prospective TA.

9. Conclusions and recommendations

All 'NGT events' must undergo a mandatory approval process before being released into the environment or brought to market. Risk assessment should (as currently requested) aim to identify the intended and unintended changes resulting from the technical processes of genetic engineering, and should evaluate their potential to cause adverse effects on health and the environment. The differences between naturally occurring processes (or conventional breeding) and NGTs may be easily overlooked, but nevertheless can have serious consequences. In this context, direct and indirect effects which may be immediate, delayed or cumulative have to be taken into account.

Information to track and trace the NGT-GMOs have to made available and measures to enable consumer choice and coexistence with organic and GE free food production should be enforced.

Furthermore, a comprehensive and prospective technology assessment is essential prior to use in an agricultural setting in order to address systemic risks to biodiversity, socio-economic impacts and effects in regard to sustainability. There are no mechanisms in place and no data available to distinguish 'empty promises' from 'real benefits'. While NGTs, e.g. CRISPR/Cas, have huge potential to alter the genome, this potential does not easily translate into real benefits.

Technology assessment should be carried out in accordance with the precautionary principle and, at the same time, evaluate the actual need to apply the technology and also consider alternatives that could be made available. The single overarching principle should be to generally restrict releases of NGT-GMOs into the environment to avoid e.g. passing potential tipping points causing irreversible damage to ecosystems.

Abbreviations

The following abbreviations are used frequently throughout the report:

CRISPR/Cas: Clustered regularly interspaced palindromic repeats/CRISPR associated

GMO: Genetically modified organisms (in the context of the report GMOs are only considered in so far as they are genetically engineered and regulated by Directive 2001/18/EC)

Indels: Insertions and deletions on the level of the genome

NGT: New genomic techniques (also New GE or genome editing)

Omics: Methods such as genomics, transcriptomics, proteomics, metabolomics which can be used to investigate changes on the level of the genomes, the cells or the organisms

SDN: Site-directed nucleases

SynBio: Synthetic biology (which is primarily a concept for engineering new life forms)

TALENs: Transcription activator-like effector nucleases

WGS: Whole genome sequencing

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