Am I Regulated?

The US example: why new methods of genetically engineering crop plants need to be regulated

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Summary

New methods of genetic engineering, also known as genome editing, are increasingly at the centre of controversial public debate. One crucial question is, how the risks of organisms resulting from this methods should be assessed.

In the EU, all genetically engineered organisms must undergo a mandatory risk assessment. In the USA, on the other hand, there are no such legal requirements, instead individual cases are registered at the US Department of Agriculture resp. the APHIS division (Animal and Plant Health Inspection Service) to assess whether they need to be regulated.

For the purposes of this report, we have chosen organisms already registered with the APHIS division of the US Department of Agriculture (USDA), which offers a program titled "Am I Regulated?". The applications filed at APHIS are especially relevant because some of the organisms (mostly plants) are intended for cultivation in the near future, and for use in food and feed production.

The following questions were included:

› Which organisms and technical methods were considered?
› What was examined and what was the outcome?
› What were the conclusions in general for risk assessment and what are the consequences for EU regulation?

Up until end of 2018, APHIS received more than 70 applications from large companies, research institutions and universities. 22 applications were specifically for new genetic engineering techniques (also known as 'genome editing'). The applications all involved the use of nucleases such as CRISPR/Cas and TALEN to change the genomes of 21 plants and 1 mushroom. In particular, the use of the nuclease CRISPR/Cas has increased substantially in the past few years. With this method, no new genes were inserted in the genome, instead natural genes were knocked out or changed in their structure.

The plant species listed in APHIS are pennycress, green foxtail, potatoes, camelina, alfalfa, maize (corn), rice, soybeans, tobacco, tomatoes and wheat. The intended traits can be categorised as follows: changes in oil composition (5 examples); other changes in plant composition (5 examples); food production criteria such as harvest, transport and processing (4 examples); improved resistance to plants diseases (3 examples); environmental stress (1 example) and higher yield (1 example).

APHIS gave all applications non-regulated status. None of the applications were referred for further more detailed assessment. APHIS only has very little leeway in its decision-making. In its notifications the authority states that genetically regulated organisms can only be regulated, i.e. undergo more detailed assessment resp. not be released if the plants are classified as a pest resp. pathogen for plant diseases (plant pest), or have the potential to become noxious weeds.

As shown in the filed documents, in most cases the process of genetically engineering the plants is carried out in several steps and includes pre-existing genetic engineering methods: in a first step, older methods such as the 'gene gun' (biolistic method) or gene transfer via agrobacterium tumefaciens are commonly used. These methods do not allow the targeted insertion of additional genes, only random insertion. This first step is necessary to introduce the DNA sequence for the nuclease into the plant genome to establish the preconditions for the nuclease to be activated in the cells. The outcome of this first step are transgenic plants with DNA sequences originating from microorganisms and other organisms - the genes have been inserted randomly into the genome often with several and flawed copies. It is only in a second step that the nucleases intended to target specific locations in the genome are formed in the cells and ultimately lead to the desired changes; the previously inserted gene constructs provide the necessary preconditions for this process.
The exact intended characteristics cannot always be precisely determined. In many of the documents no information is provided because the precise description of the targeted genes is categorised as confidential business information (CBI). As a rule, it is also difficult to find information on the progress of development – it does however appear that applications are filed at early stage. Generally, it has to be assumed that by no means will all of the plants registered at APHIS come on to the market. On the other hand, DowDuPont (Corteva) and Calyxt have announced to investors that some specific plants will be on the market very soon.

In order to examine the usefulness and reliability of the US system, the report provides a description of the characteristics of the new methods of genetic engineering; in addition, we include an overview of the differences between conventional breeding and pre-existing methods of genetic engineering.

Essentially, conventional breeding is always based on a wide range of genetic and biological diversity found in natural populations, as well as in all previously bred plant and animal varieties and breeds. In addition, new mutations happen continually and specific triggers can speed up the occurrence of mutations. Not all of these mutations are considered beneficial. In order to achieve the desired results, breeders screen natural populations and previously bred varieties for specific traits. Subsequently, plants are chosen and then grown and crossed to achieve an optimal combination of genetic information. The natural mechanisms of inheritance and gene regulation cannot be bypassed with this method.

Genetic engineering on the other hand uses direct technical and targeted intervention to establish new traits; whereby additional genetic changes are not desired but regarded as unintended effects. These technical interventions bypass natural biological mechanisms governed by evolution, inheritance and gene regulation, and can therefore be much faster than conventional breeding. Since genetic engineering intervenes directly in the genome, the resulting plants and animals can be very different to those from conventional breeding. Therefore, it is necessary to treat these organisms with caution before any environmental releases take place or they are approved for use in food production.

The differences to conventional breeding are very clear despite the reduced amount of information provided by APHIS applicants. The following examples illustrate the differences:

- simultaneous manipulation of several genes,
- simultaneous manipulation of several gene copies,
- separation of genes that are naturally only transferred together.

In the context of this evaluation, it is clearly evident that the APHIS system is unsuitable to sufficiently assess the risks of organisms developed with new methods of genetic engineering and changed in their genome. Three categories can be identified:

1. APHIS ignores the differences between conventional breeding and genetic engineering. In general, the risks associated with genetically engineered organisms by no means solely depend on whether or which new genes are inserted. The removal of gene copies and specific patterns of genetic or epigenetic changes can alter the biological characteristics of plants in other ways than might be expected from conventional breeding. As a result, plants and other organisms can emerge that are not only changed in their gene structure but which, due to their unexpected biological traits and associated risks, are clearly different to plants from conventional breeding. APHIS completely ignores this aspect in its opinion.
2. APHIS only takes into account the intended effects. By doing so, APHIS overlooks that plants engineered with a combination of `gene gun' and CRISPR can show many undesirable changes in their genome, even if no more transgenes can be found in the genome. Moreover, APHIS does not take into account unintended changes in the genome that are often caused by incorrect use of the gene scissors. The authority further overlooks that unexpected effects might only become apparent in interaction with the environment or after several generations.

3. The APHIS system treats too much relevant information as confidential business information (CBI). This prevents informed public debate and makes in-depth risk assessment by independent scientists either very difficult or practically impossible.

Specific gaps in risk assessment are, in particular:

- unintended changes in plant metabolism,
- interaction between the genome and the environment,
- effects on following generations.

There is no scientific justification for general assumptions that conclude on the overall safety of genetically engineered organisms simply on the basis that no additional genes are inserted. The extent of the actual risks needs to be assessed in each case. Preventative measures must therefore be implemented or prohibitions imposed for plants that either have, or could develop, the potential to spread.

Therefore, the risk assessment of organisms developed with the new methods of genetic engineering should take the following criteria into account:

- the whole pattern of genetic changes and their effects need to be considered, including the impact on cells and organisms;
- if, in specific cases, it is assumed that the results of genome editing cannot be distinguished from those of conventional breeding, then comparative data must be requested, including whole genome sequencing data;
- data from so-called omics techniques must also be provided to assess unintended changes in the genome that, for instance, might have been caused by `gene-gun’ methods (biolistic methods) or by the nucleases themselves;
- omics data are also necessary to assess changes in the transcriptome, the proteome and the metabolome in order to assess the effects of gene changes in the organism;
- the genetically engineered organisms should be exposed to wide range of defined environmental stress conditions to, in particular, test their response to climate change or pathogens;
- effects on the associated microbiome (in particular soil organisms) must be taken into consideration;
- the assessment of risks from consumption of respective products should also focus on the microbiome in the gastrointestinal tract;
- if plants are cultivated, then effects on the food web have to be taken into account;
- likewise potential adverse effects on pollinators, beneficial and protected species;
- effective measures need to be implemented and prohibitions imposed to prevent the uncontrolled spread of genetically engineered organisms into the environment.
Summary

In addition:

› all relevant genomic data that provide information on the exact genetic changes should be made publicly available in databases;

› labelling should be mandatory and measures should be taken to protect conventional production in order to protect freedom of choice for breeders, farmers and consumers;

› state run programs should be initiated with the participation of civil society (especially nature protection-, environmental- and consumers’ rights groups) to agree on goals in research and development as well as concomitant research in risk assessment.
1. Introduction

Even in the short time since the development of new genetic engineering techniques, a huge number of plants and animals have already been gene-edited using, in particular, so-called gene scissors (nucleases) CRISPR and TALEN. Papers have been published describing experiments with flax, barley, potatoes, lucerne, maize, poplars, petunias, rice, lettuce, soya, sorghum, tomatoes, wheat and citrus trees (Tang & Tang, 2017; Zhang, 2018). Genome editing and nuclease techniques have also been applied in experiments with livestock, such as pigs, cattle, sheep (Tan et al., 2016) and poultry (Wang et al., 2017) as well as with insects, such as bees, flies, mosquitos and species of lepidoptera. (Taning et al., 2017). More recent publications on tomatoes (Zsögön et al., 2018) and wheat (Sanchez-Leon et al., 2018) describe an increasing number of complex changes, involving several genes being targeted simultaneously.

In most cases naturally occurring genes are knocked out; in other cases gene functions are changed or new genes inserted. There are several factors which can influence the effectiveness of the new genetic engineering techniques, such as plant species and genome complexity (Hilscher et al., 2017; Zhu et al., 2017). For example, Arabidopsis thaliana is a plant species that is widely used in genetic engineering experiments because it has a relatively small genome that is therefore comparatively less complex. In contrast, crop plants such as maize, wheat, oilseed rape and sugar beet have a genome that is much more complex, with several additional sets of chromosomes.

In 2018, the European Court of Justice ruled that the new methods of genetic engineering (genome editing) must undergo an approval process according to existing GMO regulation 2001/18, even if no additional genes are inserted. However, breeding methods which, at the time when the regulation was adopted, were already being applied and considered safe, were deemed to be traditional and therefore excluded from regulation.

This report presents science-based reasons why organisms that have been genetically engineered with the new techniques and changed in their genome should undergo an approval process, even if no new additional genes can be found in their genome.

As a starting point, we have chosen organisms already registered in the US. The US Department of Agriculture (USDA) with its Animal and Plant Health Inspection Service (APHIS) offers a program titled “Am I Regulated?” that can be used by companies and other applicants at an early stage of development to obtain an official classification to find out whether, from the perspective of the USDA, their genetically engineered organisms need to undergo more detailed risk assessment, or if they can be used without further regulation.

› The applications filed at APHIS are especially relevant in this report because:
› some of the organisms (mostly plants) are intended for cultivation in the near future and for use in food and feed production;
› according to the applications, in most cases these organisms do not inherit additionally inserted DNA sequences. Consequently, this list also includes plants discussed in the context of the EU court decision.

2. Genome editing and nucleases – a short introduction

Genome editing or genome editing can be categorised under new methods of genetic engineering that are more precise and targeted than previous methods. The most important tool in this category are nucleases that are also known as gene scissors.

2.1 Nucleases

Nucleases are proteins (enzymes) that can cut open DNA – therefore the name gene scissors. Gene scissors such as zinc finger or meganucleases have been known for some time, although it was only possible to use them to “cut” the DNA at relatively few locations. In recent years, various new nucleases have been developed that are more versatile, faster and easier to use. Currently, the most important nuclease is CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats), which was first described in 2012/2013. TALEN (Transcription Activator-like Effector Nuclease) is another method that has been used for a number of years.

The nucleases are generally made up of two elements. First, site recognition to detect the specific target structures in the genome. Once the target structure has been detected, the second element, an enzyme, can ‘cut’ the DNA strands. With CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), site recognition is a kind of ‘gene probe’ that is made up of ribonucleic acid (RNA) and a protein, i.e. the enzyme that can ‘cut’ the DNA strands (see figures). The CRISPR/Cas9 system can be ‘programmed’ to target sites via its specific guide RNA sequences. Therefore, it is possible to knock-out genes, change their structure and/or insert additional DNA into the genome.

The nuclease is intended to cut through both DNA strands. This will trigger repair mechanisms in the cell that will attempt to repair the DNA. As a result, changed DNA structures (mutations) frequently develop at the locations where the nuclease has made the cut, thereby allowing gene functions to be disrupted or blocked. This is called “knock-out” or changing of natural genes. The CRISPR/Cas9 system can also be used to insert additional DNA (synthesised in the laboratory) into the genome of a cell (called “knock-in”). It can further be used to change the genome at several locations simultaneously.
2.2 Variants of the CRISPR-System

Gene scissors are often categorised in three groups according to the function of the nucleases – whereby the term SDN or Site-Directed Nucleases technology is often used:

SDN 1 technology – introduces a double strand break (both DNA strands are cut) that is then repaired via cellular repair mechanisms, whereby each strand is changed differently in its structure (non-homologous end joining, NHEJ). As a result, random mutations are created at each site via which the respective genes can be silenced.

Figure 2: Schematical diagram of changes introduced by SDN-1 (Site-Directed Nuclease-1).

Figure 3: Schematical diagram of changes introduced by SDN-2 (Site-Directed Nuclease-2).
SDN 2 technology – additional DNA is inserted with help of the nuclease. The inserted DNA serves as a repair template (matrix) and enables a homologous repair of both DNA strands (homology-directed repair, HDR). The structure of the DNA is repaired in short sequences, but not randomly changed. Natural gene functions are also thereby often deactivated. The success rate is usually lower than with SDN 1 technology.

Figure 3: Schematical diagram of changes introduced by SDN-2 (Site-Directed Nuclease-2).

SDN 3 technology – additional (longer) DNA sequences are introduced into the cells with the nuclease. The longer DNA sequences can establish new biological functions. The success rate is often very low. Many of the biotech companies would like to exclude SDN 1 and 2 technology from genetic engineering regulation.
3. Which organisms are classified as non-regulated by APHIS?

Up until November 2018, APHIS received more than 70 applications from large companies, research institutions and universities. 22 applications were specifically for new genetic engineering techniques (also known as ‘genome editing’). The applications all involved the use of nucleases such as CRISPR/Cas and TALEN to change the genomes of 21 plants and 1 mushroom. The plant species listed in APHIS are pennycress, green foxtail, potatoes, camelina, alfalfa, maize (corn), rice, soybeans, tobacco, tomatoes and wheat. APHIS gave all 22 applications non-regulated status. The Plant Protection Act (PPA) from 2000 gives USDA the authority to oversee (1) the spread of plant pests or (2) noxious weeds in order to protect the agriculture, environment, and economy of the United States. However, articles in the well-known scientific journal ‘Nature’ point out that this system cannot be the foundation for a coherent systematic regulation of genetically engineered organisms (see Waltz, 2016 und 2018).

Which genetic engineering techniques are relevant?

The 22 applications filed with APHIS for genome editing included nucleases such as zinc finger nucleases, meganucleases, TALEN and CRISPR/Cas (Chapter 2). Overall, the percentage of plants genetically engineered with CRISPR has increased substantially in the last few years. An overview is given in Table 2.

As shown in the filed documents, in most cases the process of genetically engineering the plants is carried out in several steps and includes pre-existing genetic engineering methods: in a first step, older methods such as the ‘gene gun’ (biolistic method) or gene transfer via agrobacterium tumefaciens are commonly used. These methods do not allow the targeted insertion of additional genes, only random insertion. This first step is necessary to introduce the DNA sequence for the nuclease into the plant genome to establish the preconditions for the nuclease to be activated in the cells. The outcome of this first step are transgenic plants with DNA sequences originating from microorganisms and other organisms – the genes have been inserted randomly into the genome often with several and flawed copies.

It is only in a second step that the nucleases intended to target specific locations in the genome are formed in the cells and ultimately lead to the desired changes; the previously inserted gene constructs provide the necessary preconditions for this process.

Finally, in a third step, the changed cells resp. plants are used for further breeding: in a process known as ‘segregation’ plants are selected that no longer have the gene constructs resulting from the first step of genetic engineering. The plants are then crossbred with conventionally bred plants. Thereafter, plants are chosen from the offspring which, to greatest extent possible, show the intended genetic changes in their genome brought about by the nuclease.
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3. Which organisms are classified as non-regulated by APHIS?

Table 1: Frequently applied stepwise processes using nucleases in the genetic engineering of plants (example: CRISPR/Cas, SDN1 and SDN2)

<table>
<thead>
<tr>
<th>Step and techniques</th>
<th>Purpose</th>
<th>Intended result</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Establishing the preconditions for genome editing: use of 'gene gun' or Agrobacterium tumefaciens</td>
<td>Non-targeted introduction of gene constructs mostly for the formation of the nucleases.</td>
<td>Transgenic plants resp. cells.</td>
</tr>
<tr>
<td>(2) Editing: the nuclease is activated in the cells to bring about targeted changes.</td>
<td>Genes in the plants to be either silenced (knocked out, SDN-1) or changed (SDN-2)</td>
<td>Transgenic plants with additional and targeted changes in the genome.</td>
</tr>
<tr>
<td>(3) Segregation</td>
<td>The transgenic elements inserted in the first step of genetic engineering are bred out through crossing and selection.</td>
<td>Gene edited plants with no transgenes: changes in the genome are intended to be limited to the target location of the nucleases.</td>
</tr>
</tbody>
</table>

The stepwise process (Table 1) was used for most of the plants registered at APHIS i.e. plants with a genome changed via nucleases (see Table 2). In three of the cases, a process known as ‘polyethylene glycol (PEG)-mediated transformation method’ was used. In this latter process, which can be targeted to cells with specific characteristics, the presence of the nuclease in the cells is transient and no additional genes are meant to be inserted. In seven other cases, there were no precise details about the methods used, instead they were classified as ‘confidential business information’ (CBI).

**Which applications were approved?**

So far all the applications filed at APHIS have been approved. The decisive factor in USDA approval was that the additional gene constructs inserted in the first step of the process (see Table 1) were no longer present or traceable in the plants. APHIS simply assumes that further more detailed risk assessment is unnecessary because the plants do not show any additional DNA from plant pathogens. However, this is limited to the evaluation of the intended traits only; unintended effects are not taken into account.

The first decision, which can be seen as a test case, was made in 2011: Cellectis (now Calyxt) filed an application in September 2011 that addressed the fundamental status of plants genetically engineered with nucleases and with changed genomes (in this case meganucleases). There was a prompt response from APHIS: in December 2011, the authority announced that if no additional genes were inserted in the organisms, they did not need to be regulated. Table 2 gives an overview of non-regulated food plants and one mushroom that have all been genetically engineered with nucleases, and therefore have changed genomes.

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3 PEG: polyethylene glycol mediated DNA uptake: the naked DNA is taken up by plant cells (protoplasts) via diffusion. This technique can be applied successfully with species such as tobacco plants, potatoes and also mushrooms. With this technique, the additional DNA is only transiently present in the cells and not integrated into the genome. Proteins, in this case nucleases, may be produced that can have an effect before the DNA and proteins are degraded in the cells. This is known as transient gene activity which can, nevertheless, lead to inheritable changes in the genome.
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3. Which organisms are classified as non-regulated by APHIS?

Table 2: Overview of organisms genetically engineered with nucleases and classified as non-regulated by USDA / APHIS (CBI: Confidential Business Information; PEG: polyethylene glycol (PEG)-mediated transformation method; Step 1 and Step 2: see Table 1))

<table>
<thead>
<tr>
<th>Date of publication of the decision</th>
<th>Species</th>
<th>Applicant</th>
<th>Method</th>
<th>Intended trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.12.2011</td>
<td>Not specified</td>
<td>Cellectis (now Calyxt)</td>
<td>(1) not specified (2) meganucleases</td>
<td>Not specified</td>
</tr>
<tr>
<td>08.03.2012</td>
<td>Maize (corn)</td>
<td>Dow AgroSciences (now DowDuPont / Corteva Agriscience)</td>
<td>(1) not specified (2) zinc finger nuclease</td>
<td>Reduced phytates content</td>
</tr>
<tr>
<td>28.8.2014</td>
<td>Potato</td>
<td>Cellectis (now Calyxt)</td>
<td>(1) PEG (2) TALEN</td>
<td>Not specified / CBI</td>
</tr>
<tr>
<td>05.05.2015</td>
<td>Soybean</td>
<td>Cellectis (now Calyxt)</td>
<td>(1) CBI (2) TALEN</td>
<td>Changed fatty acid composition</td>
</tr>
<tr>
<td>20.05.2015</td>
<td>Soybean</td>
<td>Cellectis (now Calyxt)</td>
<td>(1) CBI (2) TALEN</td>
<td>Changed fatty acid composition</td>
</tr>
<tr>
<td>22.05.2015</td>
<td>Rice</td>
<td>Iowa State University</td>
<td>(1) not specified (2) TALEN</td>
<td>Improved resistance to bacterial blight</td>
</tr>
<tr>
<td>12.11.2015</td>
<td>Maize (corn)</td>
<td>Agrivida</td>
<td>(1) CBI (2) meganucleases</td>
<td>Changed starch composition</td>
</tr>
<tr>
<td>15.04.2016</td>
<td>Mushroom</td>
<td>Penn State University</td>
<td>(1) PEG (2) CRISPR</td>
<td>Non browning / improved storage</td>
</tr>
<tr>
<td>18.04.2016</td>
<td>Maize (corn)</td>
<td>DuPont Pioneer (now DowDuPont / Corteva Agriscience)</td>
<td>(1) ‘gen canon’ (2) CRISPR</td>
<td>Changed starch composition (“waxy corn”) (no precise information, CBI)</td>
</tr>
<tr>
<td>15.9.2016</td>
<td>Potato</td>
<td>Calyxt</td>
<td>(1) PEG (2) TALEN</td>
<td>For improved processing (no precise information, CBI)</td>
</tr>
<tr>
<td>02.11.2016</td>
<td>Wheat</td>
<td>Calyxt</td>
<td>(1) ‘gen canon’ (2) TALEN</td>
<td>Improved resistance to powdery mildew</td>
</tr>
<tr>
<td>02.12.2016</td>
<td>Potato</td>
<td>Simplot</td>
<td>(1) Agrobact. tumefaciens (2) TALEN</td>
<td>Improved storage</td>
</tr>
<tr>
<td>07.4.2016</td>
<td>Green foxtail</td>
<td>Danforth Center</td>
<td>(1) Agrobact. tumefaciens (2) CRISPR</td>
<td>Change in timing of flowering for higher yield</td>
</tr>
<tr>
<td>29.8.2017</td>
<td>Camelina</td>
<td>Yield 10</td>
<td>(1) Agrobact. tumefaciens (2) CRISPR</td>
<td>Changed oil composition (no precise information, CBI)</td>
</tr>
<tr>
<td>25.9.2017</td>
<td>Alfalfa</td>
<td>Calyxt</td>
<td>(1) not specified (2) TALEN</td>
<td>For improved digestibility (no precise information, CBI)</td>
</tr>
<tr>
<td>16.10.2017</td>
<td>Soybean</td>
<td>USDA</td>
<td>(1) Agrobact. tumefaciens (2) CRISPR</td>
<td>Knock-out of two genes that are assumed to affect stress and salt tolerance</td>
</tr>
<tr>
<td>29.12.2017</td>
<td>Tobacco</td>
<td>Universität North Carolina</td>
<td>(1) Agrobact. tumefaciens (2) meganucleases</td>
<td>Reduced nicotine content</td>
</tr>
</tbody>
</table>
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3. Which organisms are classified as non-regulated by APHIS?

<table>
<thead>
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<th>Applicant</th>
<th>Method</th>
<th>Intended trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 20.3.2018</td>
<td>Wheat</td>
<td>Calyxt</td>
<td>(1) not specified</td>
<td>For improved nutritional quality (exact gene and phenotype, CBI).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2) TALEN</td>
<td></td>
</tr>
<tr>
<td>20 14.5.2018</td>
<td>Tomato</td>
<td>University of Florida</td>
<td>(1) <em>Agrobact. tumefaciens</em></td>
<td>For easier harvesting</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2) CRISPR</td>
<td></td>
</tr>
<tr>
<td>21 8.6.2018</td>
<td>Pennycress</td>
<td>Illinois State University</td>
<td>(1) <em>Agrobact. tumefaciens</em></td>
<td>Changed oil composition (increased number of changed genes) (exact gene and phenotype, CBI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2) CRISPR</td>
<td></td>
</tr>
<tr>
<td>22 7.9.2018</td>
<td>Camelina</td>
<td>Yield 10</td>
<td>(1) <em>Agrobact. tumefaciens</em></td>
<td>Changed oil composition (nine target genes changed) (Exact gene and phenotype, CBI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2) CRISPR</td>
<td></td>
</tr>
</tbody>
</table>

As the table shows, in recent years there has been a considerable increase in the percentage of plants genetically engineered with CRISPR. The second most important genome editing method is TALEN – this is a nuclease method in which Calyxt in particular specialises. In most cases, as shown in Table 1, older non-targeted e.g. ‘gene gun’ methods were used in addition to the new methods of genetic engineering. In almost all the cases it appears that the nucleases were just used to knock out natural gene functions (SDN1). In at least one case (No. 18, maize), additional DNA was introduced as a template for the repair mechanism (SDN2) - this would not be considered a transgene since it was synthesised from a DNA sequence from the same species.

According to the information listed by APHIS, the intended traits can be categorised as follows: changes in oil composition (5 examples); other changes in plant composition (5 examples); food production criteria such as harvest, transport and processing (4 examples); improved resistance to plants diseases (3 examples); environmental stress (1 example) and higher yield (1 example).

However, the exact intended characteristics cannot always be precisely determined. In many of the documents no information is provided since the precise description of the targeted genes is classified as confidential business information (CBI).

As a rule, it is also difficult to find information on the progress of development – it does however appear that applications are filed at early stage. So, for example, the applicants in a USDA project for the development of a soybean with two knock-out genes (No. 16) state that this is still at an early experimental stage. According to the applicant, they first of all want to investigate the effect of the silenced genes. Generally, it has to be assumed that by no means will all of the plants registered at APHIS come on to the market.

On the other hand, DowDuPont (Corteva) and Calyxt have announced to investors that some specific plants (amongst others, ‘waxy corn’ and ‘high oleic soybean’) will be on the market very soon. Indeed, in 2018 Calyxt started growing soybeans in the US that have a changed oil composition; and in 2019 it plans to increase the crop acreage to around 14,000 hectares.⁴

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4. Differences to conventional breeding

The following sections provide an overview of some general differences between the new genetic engineering techniques and conventional breeding. These differences will be later discussed using the examples given in Table 2.

4.1 Not every genetic change is due to genetic engineering

As mentioned, breeding methods used over a longer period of time and considered safe at the time of adoption of EU regulation 2001/18, are regarded as ‘conventional’ and therefore excluded from regulation. These conventional methods include mutagenesis, which uses spontaneous or induced non-targeted mutations in the genome. According to Directive 2001/18, these plants are also considered to be ‘genetically modified organisms’ (GMOs). However, the term in this sense is used very generally and could be applied to any method of breeding. In contrast, the newer methods of genetic engineering that bypass natural mechanisms of gene regulation and inheritance are subject to EU regulation and approval processes.

Essentially, conventional breeding is always based on a wide range of genetic and biological diversity found in natural populations, as well as in all previously bred plant and animal varieties and breeds. In addition, new mutations happen continually and specific triggers can speed up the occurrence of mutations. Not all of these mutations are considered beneficial. In order to achieve the desired results, breeders screen natural populations and previously bred varieties for specific traits. Subsequently, plants are chosen and then grown and crossed to achieve an optimal combination of genetic information. The natural mechanisms of inheritance and gene regulation cannot be bypassed with this method.

In particular with plants, additional ‘tricks’ can be used to increase genetic diversity e.g. by exposing the seeds to specific chemicals to increase the natural rate of mutation. This process is known as mutation breeding (mutagenesis), which, in a first step, enhances genetic diversity. The plant cells react to non-specific external stress factors. The process of conventional mutagenesis has been used in plant breeding since the mid-twentieth century.

It is important to understand that, taken as a whole, the results of mutagenesis are not totally random. They are governed by various biological mechanisms of evolution, inheritance and gene regulation which, for example, ensure that some specific genome locations are more frequently changed than others. Ultimately, breeding through mutagenesis creates greater genetic diversity, but the desired traits are not brought about by direct technical intervention. It is only through crossing and selection of plants and animals that clearly have the desired traits that a new variety can emerge from biodiversity. This process is time-consuming and requires careful choice by breeders. In many cases, unintended effects can be eliminated during this long process.

Genetic engineering on the other hand uses direct technical and targeted intervention to establish new traits; whereby additional genetic changes are not desired but regarded as unintended effects. These technical interventions bypass natural biological mechanisms governed by evolution, inheritance and gene regulation, and can therefore be much faster than conventional breeding. Since genetic engineering intervenes directly in the genome, the resulting plants and animals can be very different to those from conventional breeding. Therefore, it is necessary to treat these organisms with caution before any environmental releases take place or they are approved for use in food production (see below).
The US example: why new methods of genetically engineering crop plants need to be regulated

4. Differences to conventional breeding

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Breeding/Mutagenesis</th>
<th>New genetic engineering methods / genome editing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aims</td>
<td>Random mutagenesis or mutation-based breeding increases the range of genetic variations in the genome of plants within shorter periods of time than would normally be the case. The increase in genetic variation is then the starting point for selection and further crossing and selection.</td>
<td>Genome editing does not aim to increase the range of genetic variations in the genome. The intention is to introduce specific changes in the genome.</td>
</tr>
<tr>
<td>Depth of intervention</td>
<td>Methods of conventional breeding always work with the cell or organism as a whole.</td>
<td>Genetic engineering intervenes directly in plant DNA. In each case, material synthesised in the laboratory is physically inserted into the cells (DNA, RNA, enzymes).</td>
</tr>
<tr>
<td>Natural gene regulation</td>
<td>The results of mutagenesis are dependent on various factors. These include the kind of stimulant used to trigger the mutations, as well as cell mechanisms such as gene location, repair mechanisms and other elements of gene regulation.</td>
<td>The desired effects can be achieved through bypassing natural gene regulation and rules of inheritance.</td>
</tr>
<tr>
<td>Patterns of genetic change in the genome</td>
<td>Plants in particular often have a redundant genome i.e. genetic information that is repeated. Methods of conventional and mutation-based breeding do not generally change all gene sequences with the same genetic information at the same time.</td>
<td>In most cases, all sequences / gene clusters with the same genetic information are all changed at the same time.</td>
</tr>
<tr>
<td>Epigenetics</td>
<td>There are some specially preserved areas in the genome in which there is either no, or rarely, any natural mutation, and which are only slightly affected by evolutionary processes.</td>
<td>Even the specially preserved areas in the genome can be accessed and changed by using CRISPR/Cas. Whereby efficacy can be variable in each case.</td>
</tr>
<tr>
<td>Repair mechanisms in the genome</td>
<td>Original gene sequences often still exist alongside the newly mutated versions. These can serve as templates for repair processes.</td>
<td>If DNA changed by CRISPR/Cas technology is changed back to its original status by cell repair mechanisms, the nuclease will again recognise its target region and stay active until all the original DNA structure has been destroyed.</td>
</tr>
<tr>
<td>Multiple genetic changes</td>
<td>Mutagenesis generally leads to changes at several gene locations. The result of the change is not specific for each method.</td>
<td>Gene editing makes it possible to change several of the same, or different genes, at the same time. These changes create specific new combinations of genetic information. Even when the individual changes only affect a small sequence of DNA, in combination, the changes can lead to substantial changes in the biological characteristics of the organism.</td>
</tr>
<tr>
<td>Traceability</td>
<td>In general, the plants are identifiable by one or several gene locations. However, the pattern of the genetic changes is not specific.</td>
<td>The plants are often identifiable through a specific pattern (fingerprint) of the genetically engineered changes.</td>
</tr>
</tbody>
</table>
4.2 The role of gene regulation

Equating conventional mutagenesis with genetic engineering technologies is scientifically misleading. This can be seen very clearly if, for instance, a closer look is taken at the natural mechanisms of gene regulation. These may be seen as natural ‘rules’ for changes in the genome that cannot be overstepped:

- Epigenetic mechanisms (which are crucial for gene regulation in general) can, amongst others, cause DNA in some regions of the chromosomes to be packed very densely and therefore be less active. Chromatin is also involved in these functions and has effects on the emergence of mutations: these occur in some regions of the genome more seldom than others depending on the structural composition of the DNA. (Makova & Hardison, 2015). Some of these effects can to an extent be observed in the application of nucleases (Cho et al., 2017; Daer et al., 2017).

- It is well known that in recombinations resulting from the cross breeding of plants, the respective changes occur in specific ‘hotspots’ while in other regions they hardly occur at all. (Choi et al., 2018; Si et al., 2015). Therefore, the probability of a specific recombination of genes via sexual reproduction can vary substantially depending on the gene location.

- Repair and steering mechanisms can be observed during meiosis (cell division) that influence the pattern of spontaneous and induced mutations. For example, some regions in the genome are repaired more frequently than others. (Belfield et al., 2018).

- Plants can reproduce chromosomes or specific gene sequences. These act as ‘backup’ copies in the genome, i.e. there are several copies of many gene sequences present in the cells. If one copy is lost via mutations, the cell can still use the other copies. (Semon & Wolfe, 2007).

- Cell-specific adaptation mechanisms include so-called transposons, these enable gene sequences within the genome to be copied to another location; whereby various mechanisms influence the frequency and location of the genes – depending on the type of transposon (for example see Vicent & Casacuberta 2017).

- If mutations occur, their biological effect very often depends on the genetic background, i.e. the genome as a whole (see Chandler et al., 2013; Mullis et al., 2018). This means that specific effects can be weakened or strengthened by gene regulation and the interaction of genes and their copies.

The new methods of genetic engineering can bypass these natural mechanisms of gene regulation either wholly or partially, and the underlying mechanisms are fundamentally different from those of conventional breeding. Consequently, the results and risks are very different to outcomes from conventional breeding (see below). Interestingly, biotech companies themselves clearly distinguish between conventional (mutagenesis) breeding and genome editing in their technical descriptions. Contrary to what the public are being told, Monsanto, for instance, clearly regards CRISPR/Cas applications as a method of genetic engineering and not just plant breeding. For example, in several Monsanto patents applications it states that (see e.g. WO2017044744, page53): “Exemplary genome engineering techniques include meganucleases, zinc-finger nucleases, TALENs and CRISPR/Cas9 systems (...). A plant or seed disclosed herein can also be subject to additional breeding using one or more known methods e.g. pedigree breeding, recurrent selection, mass selection, and mutation breeding.”
4.3 APHIS registrations reveal differences to conventional breeding

The differences to conventional breeding are very clear despite the reduced amount of information provided by APHIS applicants. The following examples illustrate the differences:

› simultaneous manipulation of several genes,
› simultaneous manipulation of several gene copies,
› separation of genes that are naturally only transferred together.

Simultaneous targeting of several different genes (multiplexing)

The company Yield 10 intends to change three genes (18 genome locations in total) in camelina (Table 2, No. 22) using CRISPR/Cas to specifically change the oil composition in the plants. The technique applied here, also known as multiplexing, involves the use of the gene scissors CRISPR/Cas together with multiple different guide RNAs. It allows simultaneous changes in several genes and therefore the introduction of gene combinations, which are difficult or impossible to achieve through conventional breeding. Similar outcomes can be reached if the steps involved in genome editing are repeated (one after the other) several times. The probability that these gene locations would all be coincidentally changed simultaneously through conventional mutagenesis is very low indeed.

This genetic engineering method creates a pattern of gene changes that leaves an unmistakable ‘finger print’ in the genome – a kind of signature that does not happen in conventional breeding. Moreover, even if no additional genes are inserted, the effect on the overall metabolism and biological traits of the plants is substantial.

Simultaneous changes in multiple gene copies resp. gene families:

This is relevant for several organisms listed in applications registered at APHIS. Many crop plant species have a very large genome with several sets of chromosomes in multiple copies; and in which multiple copies of genes exist. For example, potatoes are known to have four and wheat six or eight sets of chromosomes. This is known as tetra-, hexa- or octoploidy. The number of gene copies is not only dependent on the number of chromosomes. In many cases, gene clusters are very often present, i.e. multiple copies of specific gene sequences are present in the chromosomes. Conventional breeding would hardly be able to influence these at all (for example, Cho et al., 2017; Sanchez-Leon et al., 2018).

Nucleases such as CRISPR or TALEN that are programmed to target specific regions in the genome, and are meant as a rule to simultaneously change all the genes that have the same (or similar) structure. If genes are repaired, the gene scissors will recognise the gene and change it again. Consequently, all the backup copies are deleted at the same time: an effect that would either be very difficult or impossible to achieve or not at all through conventional breeding. On this difference between genome editing and conventional breeding Duensing et al. (2018), for example, state that: “(…) genome editing can be targeted to a specific gene. However, few plant genes are found as single genes. (…) genome editing is adept at knocking out genes present in multiple copies. Thus, whenever a crop is found with multiple copies of the same gene knocked out, it will be almost certain that genome editing was used.”

Examples of these patterns of genetic changes are, for example, the non-browning mushroom (Table 2, No. 8) or potatoes developed by Calyxt (Table 2, No.10) and Simplot (Table 2, No.12), which have improved storability. Other examples include wheat with improved resistance to powdery mildew developed by Calyxt
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(Table 2, No. 11), tobacco with reduced nicotine content developed by the University of California, (Table 2, No. 17) and camelina with changed oil content (Table 2, No. 14). According to information provided by the applicants, all of the above involved multiple copies of a gene being changed simultaneously in different locations. In all of these cases, it can be assumed that the pattern of genetic change is different to that found in conventional breeding.

The separation of genes with ‘coupled’ traits

There is a further interesting difference that can be seen in tomatoes developed by the University of Florida: the gene that was changed ensures that the tomatoes could be picked more easily, i.e. were more easily detachable from the stalk; something that the applicant claimed had already been known for some years. There was already a random mutation here that only occurred with a change in the shape of the tomatoes, something that is only desirable for some varieties. So far it has not been possible to separate these traits because they are very close together on the chromosome and therefore always coupled. As Lin et al. (2014) show, the coupling of genetic traits after plants are crossed very often leads to a bias in the resulting genetic pattern. This is a widely observed phenomenon. For example, in the tomatoes about 25 percent of all genes are affected.

According to Duensing et al. (2018), the separation of coupled genetic traits is another important difference compared to conventional breeding: “One important difference is that some crop genes lie in low or non-recombinogenic regions of the chromosome. (…) Genome editing ensures all genes are amenable to allele replacement.” (See also Lin et al., 2014.)

4.4 APHIS ignores the differences between conventional breeding and genetic engineering

It is not immediately obvious that the APHIS opinion is incorrect in regard to single genetic changes – as described in the applications – that could emerge from spontaneous mutations. But, for example, in the case of the genetically engineered camelina registered in 2018 (Table 2, No. 22) where 18 alleles were changed simultaneously – then the difference to conventional breeding becomes very evident. The probability that all these gene locations could be simultaneously changed through conventional mutagenesis is very low to practically impossible.

Moreover, APHIS overlooks that plants engineered with a combination of ‘gene gun’ and CRISPR can show many undesirable changes in their genome, even if no more transgenes can be found in the genome. The use of ‘gene gun’ methods in the first step of the genetic engineering process (see Table 1) can often lead to significant changes in the genome (such as deletions or inversions), which will remain unnoticed if screening is only looking for the inserted gene constructs. In order to find such undesirable genetic changes, the whole genome would need to be systematically investigated (whole genome sequencing) and the result compared to the original plant genome. Epigenetic changes are also relevant in this respect (Jupe et al., 2019) and need to be investigated. However, APHIS does not request any of these data.

Furthermore, APHIS does not take into consideration unintended changes in the genome that are often caused by incorrect use of the gene scissors (Kosicki et al., 2018). These unintended changes occur, amongst others things, through nucleases being inserted in the wrong genome location (off target), or if other additional genes are inserted in the target gene (on target) (see below). The pattern shown by these unintended changes can be clearly different to that which might be expected from random mutation; this can have an effect on plant traits and therefore require more detailed investigation.
In general, the risks associated with genetically engineered organisms by no means solely depend on whether or which new genes are inserted. The removal of gene copies and specific patterns of genetic or epigenetic changes can alter the biological characteristics of plants in other ways than might be expected from conventional breeding. As a result, plants and other organisms can emerge that are not only changed in their gene structure but which, due to their unexpected biological traits and associated risks, are clearly different to plants from conventional breeding. APHIS completely ignored this aspect in its opinion.

Table 4: Some differences between conventional breeding, transgenic plants and genome editing

<table>
<thead>
<tr>
<th>Conventional breeding</th>
<th>Transgenic plants</th>
<th>Genome editing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based on a wide range of biodiversity, with subsequent selection and further crossing.</td>
<td>Changes in traits of existing varieties are supposed to be inserted without changing the overall characteristics of the plants.</td>
<td>In many cases, overall plant metabolism is changed to influence e.g. growth or composition.</td>
</tr>
<tr>
<td>New gene combinations follow the non-specific rules of evolution, inheritance and gene regulation.</td>
<td>Gene insertion from other species, bypassing the natural mechanisms of heredity.</td>
<td>The specific pattern of the new gene combinations is often very different that of conventionally bred plants.</td>
</tr>
<tr>
<td>Biological traits can be particularly distinctive, but do not overstep the potential of natural biological diversity.</td>
<td>The biological traits overstep the potential of natural biological diversity within the species.</td>
<td>The biological traits can overstep the potential of natural biological diversity.</td>
</tr>
</tbody>
</table>
5. Which risks are relevant?

APHIS only has very little leeway in its decision-making on the risks of genetically engineered plants within the framework of the Plant Protection Act (PPA). In its notifications the authority states that genetically regulated organisms can only be regulated, i.e. undergo more detailed assessment resp. not be released if:

1. the plants are classified as a pest resp. pathogen for plant diseases (plant pest);
2. or have the potential to become noxious weeds.

In short, APHIS is responsible for protecting plant health as well as US agriculture and its natural resources. The APHIS examination process only, for example, asks whether genes from specific microorganisms that could trigger plant diseases were inserted in the plants. If no such genes were inserted, then no further investigation is necessary. As yet, it is almost exclusively gene edited organisms that have been registered under the ‘Am I regulated?’ program; according to the applications no additional genes were inserted and all applicants were told that their products did not need to be regulated.

The second criterion, noxious weeds, is somewhat more differentiated: for green foxtail (Table 2 No. 13), the authority warns that it can cross with some other weeds, and that every effort should be made to avoid this. For pennycress (Table 2 No. 21) and camelina (Table 2 No. 22), the authority notes that these can themselves become weeds resp. cross with other weeds and therefore farmers, amongst others, should be made aware of this problem. However, with such vague and hardly verifiable recommendations, the authority has in effect dispensed with regulation even in these cases.

This kind of superficial preliminary assessment arises from the legal mandate of the authority and has serious consequences for companies and other applicants: if released, products are considered to be safe for cultivation and can be marketed.

However, some specific products need to undergo further assessment by the US Environmental Protection Agency (USEPA) or the US Food and Drug Administration (FDA). These assessments follow on a case by case basis and depend essentially on the intended traits of the respective organisms. Whether the organisms already released by APHIS so far are required to undergo further assessment remains to be seen.

Apart from this, there are serious gaps in APHIS risk assessment from the point of view of US agriculture:

1. **Unintended changes in the plant metabolism**
   The APHIS approval process only includes looking at known traits of the genetically engineered plants – unexpected and unintended side effects are left aside. If, for example, there are unintended effects in plant metabolism that lead to disruption in other farming the processes (pollinators, useful plants/insects, soil life), they would probably go unnoticed. However, changes in the composition of biologically active substances can affect natural plant defence mechanisms and other interactions with the environment.

2. **Interactions between genome and environment**
   The true fitness and vitality of the plants and their potential for spread remains untested. Therefore, no predictions can be made regarding the responses of the plants to stress e.g. plant diseases or climate change. Plant diseases could spread more rapidly if plant vitality is weakened. If the plants show higher potential to spread due to unintended genetic or epigenetic effects, then the danger of these plants becoming weeds themselves would be very much higher than assumed by the authority.
3. **Next generation effects**

If the plants cross with conventionally bred varieties or wild populations, their offspring can, due to interaction with specific gene combinations or hybridisation, be significantly different to the original populations. In these cases, the potential of the plants to become weeds themselves or transfer diseases can change substantially. APHIS is aware of this problem; as stated in the Calyxt application for genetically engineered wheat (Table 2, No. 11): "(...) wheat is sexually compatible with a weedy relative, jointed goatgrass (Aegilops cylindrica). (...) Despite this low hybrid fertility, any fitness enhancing GE trait can persist and become widespread overtime in the hybrid-derived weedy populations."

Beyond the concerns of agriculture, there are further risks that have to be assessed before any conclusion can be made on the safety of the genetically engineered plants in regard to human health and the environment. Some examples:

- Changes in the composition of associated microorganisms (microbiome) can have a substantial impact on soil organisms, the food web and protected species.
- Wild populations and therefore biological diversity can be damaged if the genetically engineered organisms develop invasive traits.
- Changes in plant composition can cause unintended effects if the plants are used for food or feed. This includes people, livestock and/or associated food webs of wild populations. For example, Colombo et al. (2018) show risks for food webs that result from the extensive cultivation of genetically engineered plants such as camelina: the fatty acids in the plants can, for instance, change the growth and fecundity of the organisms that feed on them. Such effects could be carried forward into the food chain.

The actual level of damage to people, animals and the environment will depend, (amongst others) on the number of organisms released, the extent of the affected acreage and the duration of the release. Large scale cultivation of plants, even if they only triggered slight changes in experimental field trials, can over the years have a substantial detrimental effect on biodiversity and agroecosystems. This is particularly problematic when genetic changes spread to wild populations.

There is no scientific justification for general assumptions that conclude on the overall safety of genetically engineered organisms simply on the basis that no additional genes were inserted. The extent of the actual risks needs to be assessed in each case. Preventative measures must therefore be implemented or prohibitions imposed for plants that either have, or could develop, the potential to spread.

One example of what can go wrong under the current APHIS approach are (with first generation methods) genetically engineered herbicide-resistant transgenic grasses. These plants were released by APHIS without any further detailed risk assessment (Waltz, 2018). The uncontrolled spread of the herbicide-resistant genetically engineered grasses has been causing problems in the US for several years (see, amongst others, Bauer-Panskus et al., 2013). Like most genetically engineered plants, the grasses are resistant to glyphosate. The grasses have been genetically engineered to produce an additional enzyme (EPSPS). The problem: as current publications show, this enzyme not only makes the plants resistant to glyphosate, but also unexpectedly increases the potential to spread (Fang et al., 2018). Their offspring can produce more seeds and therefore spread much faster than the authority previously thought. The effect of this enzyme on the potential for spread of the plants was wrongly assessed by the authority for more than 20 years.

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6. Outlook and recommendations

In the EU, the new methods of genetic engineering are regulated under existing GMO regulation. As more experience is gathered with gene editing, it will become necessary to establish specific guidance or implementing regulations to ensure that risk assessment meets the new challenges.

In the context of genome editing, the aim is often to not only change single plant traits, but to change plant metabolism as a whole. For this reason, the current ‘comparative risk assessment’ approach (EFSA) has evidently reached its limits: and in many cases it can be very difficult or impossible to find comparative organisms. Therefore, the risk assessment of organisms developed with the new methods of genetic engineering should take the following criteria into account:

- the whole pattern of genetic changes and their effects need to be considered, including the impact on cells and organisms;
- if, in specific cases, it is assumed that the results of genome editing cannot be distinguished from those of conventional breeding, comparative data must be requested, including whole genome sequencing data;
- data from so-called omics techniques must also be provided to assess unintended changes in the genome that, for instance, might have been caused by ‘gene gun’ methods (biolistic methods) or by the nucleases themselves;
- omics data are also necessary to assess changes in the transcriptome, the proteome and the metabolome in order to assess the effects of gene changes on the organism;
- the genetically engineered organisms should be exposed to wide range of defined environmental stress conditions to, in particular, test their response to climate change or pathogens;
- effects on the associated microbiome (in particular soil organisms) must be taken into consideration;
- the assessment of risks from consumption of respective products should also focus on the microbiome in the gastrointestinal tract;
- if plants are cultivated, then effects on the food web have to be taken into account;
- likewise potential adverse effects on pollinators, beneficial and protected species;
- effective measures need to be implemented and prohibitions imposed to prevent the uncontrolled spread of genetically engineered organisms into the environment.

In addition:

- all relevant genomic data that provide information on the exact genetic changes should be made publicly available in data bases;
- labelling should be mandatory and measures should be taken to protect conventional production in order to protect freedom of choice for breeders, farmers and consumers;
- state run programs should be initiated with the participation of civil society (especially nature protection-, environmental- and consumers’ rights groups) to agree on goals in research and development as well as concomitant research in risk assessment.
The US example: why new methods of genetically engineering crop plants need to be regulated

Resources


The US example: why new methods of genetically engineering crop plants need to be regulated

**Resources**


