TESTBIOTECH Background 12 - 4 - 2021

What Members of the European Parliament should consider when discussing New Genetic Engineering (New GE) with STOA

Testbiotech e. V.
Institute for Independent Impact Assessment in Biotechnology

Table of Contents

Summary

The Panel for the Future of Science and Technology (STOA) is a service providing committees and other parliamentary bodies in the European Parliament with independent, impartial and accessible information relating to developments in science and technology, including the opportunities they offer alongside the risks and ethical implications.[1] New technological developments in biotechnology, such as the CRISPR/Cas gene scissors, are part of its mandate. On 15 April 2021, STOA will hold an online conference on the genome editing of plants.[2]

STOA carried out a stakeholder consultation on genome editing in plants ahead of the event. There is however no sign that it will publish any of the submitted comments. In the interests of transparency, Testbiotech has therefore decided to publicize its input to this consultation as a backgrounder. Our input shows that current GMO regulation is clearly sufficient in regard to genome edited plants.

1 https://www.europarl.europa.eu/stoa/en/about/history-and-mission
2 https://www.europarl.europa.eu/stoa/en/events/details/the-challenges-of-genome-editing-in-plan/20210301WKS03261

At the same time, Testbiotech has some concerns that the STOA conference programme might not be sufficiently well-balanced: according the draft programme,[3] there are several members of an international lobby organisation (PRRI) among the speakers who are known for their advocacy of the biotech industry. In addition, the keynote speaker is known to have filed patent applications covering gene editing that are licensed exclusively to a large biotech company (Corteva/ DowDupont). These concerns regarding the speakers were communicated to STOA members in a joint Testbiotech and Corporate Europe Observatory (CEO) letter sent before the conference. The findings are presented in the second part of this backgrounder.

A section relating to gene edited plants in a recent European Group on Ethics in Science and New Technologies (EGE) report will be presented during the conference. Testbiotech analysed this section of the report and concluded that it is not sufficiently science-based. We therefore recommend that it is reviewed by independent scientists not involved in its compilation and who are free of any interests in the application of the technology.

1. STOA consultation and Testbiotech input

STOA carried out an online survey to understand the concerns and opinions of society regarding genome-editing in plants and crops as part of its preparations for the conference. There is however no sign that STOA will publish any of the submitted comments. In the interests of transparency, we are therefore including an edited version of our input in this backgrounder. Briefly, our input demonstrates that current GMO regulation is clearly sufficient in regard to genome edited plants.

The STOA consultation asked stakeholders to give their opinion on four policy options regarding the regulation of genome edited plants:
1) No revision of the European GMO Directive. This means that the genome-edited crops will be subject to GMO legislation, including mandatory risk assessments and monitoring of these new products;
2) Changing the European GMO Directive to exempt genome-edited plants without any foreign DNA present in the product;
3) Changing the European GMO Directive to apply different levels of risk assessment in function of the type and extent of the genetic change in an organism;
4) Changing the European GMO Directive to apply different levels of risk assessment based on an evaluation of policy objectives, socio-economic and ethical considerations.

These four policy options were explained in more detail in a study by the Dutch Rathenau Institute[4], which will also be presented at the STOA conference.

Testbiotech evaluated the different policy options and came to the conclusion that, based on current scientific knowledge of the effects of genome editing on organisms and their interactions with the environment, only the first policy option provides the necessary safeguards for the protection of humans, the environment and nature. The following section of this backgrounder provides a detailed summary of scientific arguments regarding each policy option.

3 https://www.europarl.europa.eu/stoa/en/events/details/the-challenges-of-genome-editing-in-plan/20210301WKS03261
4 https://www.rathenau.nl/en/making-perfect-lives/genome-editing-plants-and-crops

1.1 Why current EU GMO regulation must be applied for plants derived from New GE

Current GMO regulation provides the necessary preconditions to assess the risks associated with New Genetic Engineering techniques ('New GE'). These conditions are needed to ensure safety and also enable transparency, labelling and freedom of choice for consumers.

Without sufficient regulation of New GE: (i) severe damage to biological diversity is not unlikely; (ii) risks to food production may be introduced and accumulate unnoticed; (iii) access to data needed for risk assessment by independent experts will not be made available; (iv) no measures can be taken against the uncontrolled spread of the organisms into the environment; (iv) no data will be available to track and trace the New GE organisms and products derived thereof and (v) agriculture and food production relying on GE free sources would no longer be protected (see also Testbiotech, 2020).

More specifically, a process-oriented risk assessment and mandatory approval process is necessary because the application of genome editing is a multistep process, with inherent and specific risks. For example, in plants, New GE is typically combined with old genetic engineering techniques ('Old GE', such as non-targeted biolistic methods or Agrobacterium transformation), to deliver the DNA for the nuclease (gene scissor) into the cells. 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 further breeding applied to remove the transgenic elements from the plant genome. At each stage of the process, such as (i) insertion of the DNA of the gene scissors into the cells, (ii) target gene recognition and cutting and (iii) cellular repair, specific unintended alterations can occur, with associated potential risks. For example, 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.

The specific unintended effects of the overall process (also due to lack of precision of the gene scissors) include off-target effects, on-target effects (i.e. large deletions, insertions, translocations, inversions around the target site), unintended integration of DNA-sequences (e.g. from plasmid DNA, DNA templates, endogenous DNA, exogenous DNA), exon skipping (e.g. causing the unintended production of new proteins from the altered genes) and alterations of the epigenome (for more details see Kawall et al., 2020).

Furthermore, the potential of genome editing procedures to generate profound changes in the metabolism of the plants needs to be considered: genome editing can induce new genotypes by combining small changes in the genome (SDN-1 applications), e.g. by multiplexing (several genes) and/or cutting multiple copies of one gene (including multiple alleles, gene families, repetitive gene sequences). CRISPR/Cas also enables alterations in parts of the genome where fewer mutations occur; these are very unlikely to occur naturally or through conventional breeding (Belfield et al., 2018; Kawall, 2019; Kawall et al., 2020; Monroe et al., 2020). Thus, genome editing can generate new genotypes in plants that go beyond those which can be derived from processes of physical or chemical mutagenesis (Rostoks, 2021; Kawall 2021).

In addition, methods for detecting the plants and derived products are needed to enable freedom of choice as well as to track and trace GE plants within the food production chain. Identification methods are also needed in order to remove the New GE plants from wild populations if there is unintended spread into the environment. The same holds true for cases where the plants have to be withdrawn from the market if, for example, adverse health effects were to occur.

Costs can be mitigated by establishing an international register with all the necessary data to track and trace the plants (Ribarits et al., 2021).

Costs for applications under current GMO regulation could be mitigated by strengthening independent risk assessment, which can deliver some answers to general questions on risks to health and the environment. Independent risk assessment is requested in Directive 2001/18 and Regulation 178/2002 provides the option to run scientific studies. For example, more recent technologies useful in investigating the plant metabolome (so-called 'omics') could be developed further (see, for example, Enfissi et al., 2021). It is important to make sure that if independent research is funded by public money, it focuses on the protection goals (health and environment) and not on developing or applying new products.

In addition, if plants with no ethical, environmental or societal benefits were to be excluded from market access (by introduction of sufficiently defined criteria), the number of applications and costs for approval process could be dramatically reduced (see below). Specific guidance for EFSA can also be introduced to specify the need for data for different categories of GE applications (see below).

However, companies must still be obliged to deliver the data necessary for process-oriented risk assessment as well as for the identification of their specific plant applications.

Current EU GMO regulation is therefore sufficient to assess the risks of plants derived from New GE. Compared to the notification process in the US, current regulation in the EU is much more science-based, much more transparent and much more suitable for controlling New GE plants generated by a tool as powerful as CRISPR/Cas.

1.2 Why genome-edited crops and plants not inheriting foreign DNA should not be exempt from GMO regulation

Changes in regulation to exempt crops and plants with no foreign DNA present in the product would ignore known and repeatedly described process-based risks associated with the new genomic techniques ('New GE') and also downplay the potential of genome editing. This is therefore not an option for proper regulation.

Exempting genome edited crops and plants where no foreign DNA is inserted would exempt all SDN-1 (and most of the SDN-2) applications from regulation. However, SDN-1 applications have the potential to induce profound changes in the metabolism of plants that go beyond those which, e.g. can be derived from processes of conventional breeding techniques, such as physical or chemical mutagenesis. Profound changes can be the result, e.g. of multiplexing which targets several genes and/or multiple copies of one gene (including gene families, repetitive gene sequences), even if no additional genes are inserted.

CRISPR/Cas also enables alterations in parts of the genome where fewer mutations occur and that are very unlikely to occur naturally or through conventional breeding (Belfield et al., 2018, Monroe et al., 2020, Eckerstorfer et al., 2019; Kawall, 2019; Kawall et al., 2020). Thus, genome editing can generate new genotypes in plants that go beyond those which can be derived from previous methods of conventional breeding (Rostoks, 2021; Kawall 2021).

If these plants are exempted from regulation, there would be no overview of which products are on the market or what genetic alterations they carry. Even in a short period of time, it can be expected that several genome edited plants with novel traits will be released into the environment. Examples of plants with new complex genotypes, but no additionally inserted genes, include rice (Shen et al., 2017), sugar cane (Kannan et al., 2018), camelina (Morineau et al., 2017), wheat (Sanchez-Leon et al., 2018) and tomatoes (Zsogon et al., 2018). Testbiotech findings are also supported in a recent EFSA opinion on plants obtained through synthetic biology, which shows why wheat described by Sanchez-Leon et al (2017) needs to undergo risk assessment (EFSA, 2021). As EFSA (2021) explains, "[…] 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." (EFSA, 2021) A recent EFSA GMO paper published by a panel member also underlines these findings (Rostoks, 2021), as does the first publication on the environmental risk assessment of specific plants (in this case *Camelina sativa*) derived from New GE (Kawall, 2021).

The combinatorial and cumulative effects of several of the New GE plants that could be introduced into the food chain and ecosystems are hardly predictable. In the absence of proper regulation, these effects cannot be monitored and the organisms cannot be retrieved from the environment if required.

In addition, it has to be taken into account that specific risks are inherent in the multistep process of New GE. These can also be caused by a lack of precision in the use of CRISPR/Cas gene scissors (the most commonly used genome editing technique). Several publications describe how CRISPR/Cas causes unintended alterations, including off-target effects, on-target effects and chromosomal rearrangements (Kosicki et al., 2018; Lalonde et al., 2017; Kapahnke et al., 2016, Haapaniemi et al., 2018; Wolt et al., 2016; Cho et al., 2014; Sharpe, 2017; Adikusuma et al., 2018; Kosicki et al., 2020; Biswas et al., 2020; Tuladhar et al., 2019; Ono et al., 2019; Leibowitz et al., 2020; Skryabin et al., 2020; Weisheit et al., 2020; Michno et al., 2020; Norris et al., 2020; Grunewald et al., 2019; Burgio et al., 2020; Liu et al., 2021). These unintended alterations can cause a variety of unexpected effects. For example, the integrity of a non-target gene may be compromised if its coding region has been 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 toxicity and allergenicity. 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).

Unintended effects can also arise from applying old genetic engineering techniques ('Old GE', such as non-targeted biolistic methods or Agrobacterium-transformation) to deliver the DNA for the CRISPR/Cas gene scissors into plant cells (Gelvin et al., 2017; Forsbach et al., 2003; Jupe et al., 2019; Makarevitch et al., 2003; Windels et al., 2003; Rang et al., 2005). These alterations can only be detected case by case using appropriate analytical tools (e.g. long-read next generation sequencing for detecting chromosomal rearrangements or whole genome sequencing for detecting off-target effects in combination with methods such as transcriptomics, proteomics and metabolomics (Burgio et al., 2020 Enfissi et al., 2021)).

1.3 Why it is not sufficient to replace current GMO regulation with a policy where the level of risk assessment is based on the level of intended genetic changes

The arguments presented above are also relevant for the discussion on what STOA calls a 'level-based policy' of risk assessment driven by the intended genetic changes. The arguments presented show that, due to the complexity of the genome editing technology and its resulting (intended and unintended) changes, it is impossible to reduce risk assessment to intended genetic changes. Instead, the unintended genetic changes resulting from the process as well as the unintended effects triggered by the intended genetic changes have to be taken into account. For this purpose, and as requested by current GMO regulation, the starting point for risk assessment always has to be the process (and the technology that was applied), irrespective of what level of genetic changes is intended.

As explained, technical tools used in 'New GE' make the genome available for changes that go beyond those which can be derived from processes of physical and chemical mutagenesis: CRISPR/Cas also enables alterations in parts of the genome where fewer mutations occur and where they are very unlikely to occur naturally or through conventional breeding (Belfield et al., 2018; Kawall, 2019; Kawall et al., 2020; Monroe et al., 2020; Testbiotech, 2020).

It is therefore the process that has to trigger mandatory risk assessment: the CRISPR/Cas mode of action can be described as a biological mutagen which drastically increases the likelihood of a mutation occurring at a respective target site(s): if the target site is restored to its original condition, CRISPR/Cas can bind and cut again, making it very likely that the target site will eventually be altered (Brinkmann et al., 2018). This is different from the processes occurring in the cell during physical or chemical mutagenesis where, in many cases, the original gene function will be restored by natural repair mechanisms. This is just one example showing how the processes of 'New GE' can overcome natural barriers that are very often an obstacle for conventional breeding methods. Consequently, it has to be assumed that the technical process is very different when compared to breeding methods with a history of safe use.

Given the complex processes and the resulting genomic alterations (unintended or intended) it is, in most cases, impossible to decide which level of risk assessment will be needed without considering the specific steps applied during genetic engineering. This is true independently of the type and the extent of genetic intervention.

Therefore, simple categorisation or notification are not an option to replace detailed risk assessment. A sufficiently robust decision on subsequent steps in risk assessment that will ultimately be necessary to demonstrate safety, can only be taken within a process-oriented risk assessment and after full molecular risk characterisation.

Some basic steps of molecular characterisation must always be applied before any decision can be taken on the necessary level of risk assessment, e.g.: (i) whole genome sequencing should be used to assess the whole pattern of intended and unintended genetic changes and their effects; (ii) omics data are necessary to assess changes in the transcriptome (RNAs), the proteome (production of proteins) and the metabolome (several levels of metabolic functions) (Enfissi et al., 2021); (iii) comparative data must be requested to provide evidence where it is assumed that the results of 'genome editing' cannot be distinguished from those of conventional breeding.

Consequently, it has to be acknowledged that any categorisation of risks can only be achieved within a process-oriented risk assessment, but cannot be applied before or used as a replacement.

Molecular characterisation is a necessary step within risk assessment that can inform subsequent steps of assessment. It must be applied to all plants derived from New GE (genome editing) before any conclusion can be drawn on their risks for health and the environment.

1.4 Why it is not sufficient to replace current GMO regulation with a policy where the level of approval system is based on societal values

A level-based policy in which approval levels are based on societal values will result in complex processes and major controversies. It can only be considered as an additional element within the approval process, but cannot replace current regulation or process-oriented risk assessment.

The first generation of transgenic plants were originally introduced by using arguments relating to sustainability, such as reduction in pesticides and benefits to consumers. However, these promises have never materialised.[5]

Even 30 years after their introduction to the market, there is still no consensus amongst industry, farmers and food producers on the overall sustainability impact of transgenic plants in food production. This experience leads us to assume that a system based on the assessment of potential benefits before market introduction, will give rise to highly complex issues and a great deal of controversy. It is unlikely that this approach could lead to broader societal agreement or even consensus.

More specifically, there seem to be two different options for such a level-based policy:
One option would be the introduction of clearly defined criteria requesting unambiguous evidence of benefits. If this were the case, we would have to assume that none of the currently authorised events (derived from Old GE) would have been allowed on the EU market.

The second option seems to be less well-defined criteria. In this instance, it would have to be taken into account that any debate would be largely centered on the interests of marketing and trading in technology as well as respective products. If this were the case, it would be unlikely that any of the plants which were really needed would be selected. On the contrary, these criteria would (again) allow the marketing of all plants which would deliver the highest profits for the interested sectors.

Both biotech engineers and patent holders have a strong interest in marketing New GE products. It may be assumed that they would try to influence the debate to convince the public of the benefits of these products. Given these conditions, it is unlikely that the selected plants would be the ones that were really needed. On the contrary, these criteria would (again) allow the marketing of all plants likely to generate sufficient profit for the biotech companies.

These problems can also be expected to apply to New GE plants with, e.g. improved tolerance to climate change stress conditions. For example, a drought-resistant transgenic maize produced by Monsanto (MON 87460) is already causing ongoing controversy in regard to its real benefits compared to maize derived from conventional breeding.

Therefore, we would not expect such a level-based policy to be suitable to replace current EU GMO regulation. It is also important to acknowledge that potential benefits must not be seen as a reason to lower safety standards. Such an option might be considered as an additional element within the approval process, but cannot replace current regulation and process-oriented risk assessment.

5 This finding also was confirmed by a study published after the consultation was conducted, see https://science.sciencemag.org/content/372/6537/81.

2. Is the conference programme sufficiently well-balanced?

The list of speakers on the programme has attracted criticism ahead of the conference. Concerns were expressed in a joint letter drawn up by the Corporate Europe observatory and Testbiotech since two of the speakers (Mr Piet van der Meer and Mr Julian Kinderlerer) are leading members of the lobby organisation PRRI (Public Research and Regulation Initiative).[6] Mr van der Meer is currently a consultant for PRRI[7] and also one of its founding members who served treasurer on its initial Board.[8] Mr Kinderlerer is a member of the PRRI Steering Committee[9]. PRRI has received funding from the biotech industry (EuropaBio, CropLife, Monsanto/Bayer, ISAAA, Syngenta Foundation)[10] and is an advocate for industry positions in international fora, e.g. the Convention on Biological Diversity (CBD).[11] Mr Kinderlerer was also affiliated to the International Society for Biosafety Research (ISBR), serving as a treasurer on its Executive Committee.[12] The ISBR board habitually consists of many industry-affiliated scientists (e.g. from the International Life Sciences Institute, ILSI) and ISBR events are sponsored by the biotech industry[13].

The keynote speaker, Mr Virginijus Šikšnys, deserves much respect for his scientific findings on CRISPR/Cas. However, it also should be taken into account that he has vested interests in the application of the technology having filed relevant patents (WO2013141680; WO2016035044; WO 2018220583). He has also issued an exclusive licence contract to Corteva (DowDupont).[14] [15]

The Corporate Europe Observatory and Testbiotech have therefore kindly invited STOA to substantially revise the conference programme in order to avoid the appearance of bias and misconception. In a promising sign, additional speakers have been added to the panel since the letter was sent. However, there are still questions to be answered about whether the experts mentioned above are the best to present the main findings or to introduce the technology.

These concerns are supported in a recent CEO study which shows that the biotech industry is waging an ongoing battle to have New GE excluded from European GMO regulation.[16] This push in favour of the biotech industry also includes PRRI experts and scientists who have filed patents on the technology.

6 https://prri.net
7 https://prri.net/secretariat
8 https://web.archive.org/web/20050408212727/http://pubresreg.org/Members/Kim/foundation
9 https://prri.net/steering-committee
10 https://prri.net/about-prri-donate
11 https://corporateeurope.org/sites/default/files/attachments/print_version_biosafety_in_danger.pdf, https://corporateeurope.org/en/food-and-agriculture/2008/06/prri-are-these-public-researchers
12 https://web.archive.org/web/20060721192559/http://www.isbr.info/executive/
13 https://web.archive.org/web/20210116170733/https://isbr2019.com/index.php/sponsors-m/sponsors
14 http://www.prweb.com/releases/dupont-pioneer-seed/vilnius-university-cas9/prweb12804075.htm
15 https://www.nature.com/articles/nbt0116-13.pdf
16 https://corporateeurope.org/en/2021/03/derailing-eu-rules-new-gmos

3. How to interpret the EGE findings?

According to the STOA conference programme, Mr Kinderlerer will present the findings from a recent European Group on Ethics in Science and New Technologies (EGE) report, which only recently published its opinion on New GE (Genome Editing)[17]. The report looks at New GE applications in humans, animals and plants. A Testbiotech analysis of the report found that the section on plants lacks the necessary balance and scientific accuracy. Testbiotech has therefore criticised the EGE for presenting conclusions on risks associated with genetically engineered plants without sufficient scientific evidence.[18]

The EGE incorrectly claims, for example, that there is no evidence of adverse environmental effects arising from the cultivation of transgenic plants. The report does not sufficiently explain the differences between traditional breeding and genome editing and, in addition, does not show the specific risks associated with the respective New GE techniques. The report also takes a very one-sided approach in regard to the cost of the approval process and the implications of patenting.

In summary, the potential benefits are disproportionately emphasised in comparison to the risks than can be justified from the scientific evidence. Consequently, the EGE does not fulfill the high standards to which it should adhere in its high ranking publications.

Testbiotech finds this regrettable since we are also aware that other sections of the report are much more balanced in regard to the underlying science and conclusions. One possible reason for the difference in quality of these specific sections seems to be that there is only one expert at the EGE who has worked on plants thus far, i.e. Mr Kinderlerer. As mentioned above, this expert is also a member of the lobby organisation, PRRI, which is supporting the biotech industry in its fight to have New GE excluded from European GMO regulations. It would therefore have been more appropriate to choose a different expert to present and interpret the EGE findings.

4. Conclusions

Testbiotech has clearly shown that current GMO regulation is sufficient for genome edited plants. It cannot be replaced by a system which excludes plants from risk assessment that do not inherit foreign DNA. In addition, so-called ‘level-based’ assessments are not suitable alternatives to the current system.

At the same time, Testbiotech is concerned that the conference programme might not be sufficiently well balanced. As shown in this backgrounder, several of the invited speakers are members of the international lobby organisation (PRRI), who are known for their advocacy of the biotech industry. Furthermore, the keynote speaker is known to have filed patent applications covering genome editing, which appear to be licensed exclusively to a large biotech company (Corteva/DowDupont).

Finally, a section of a recent European Group on Ethics in Science and New Technologies (EGE) report will be presented during the conference. This section of the report is concerned with gene editing in crop plants. However, as the Testbiotech analysis shows, this section in the EGE report is not sufficiently science-based. Therefore, we recommend that this section of the report is reviewed

17 https://op.europa.eu/en/web/eu-law-and-publications/publication-detail/-/publication/6d9879f7-8c55-11eb-b85c-01aa75ed71a1

18 www.testbiotech.org/en/news/european-group-ethics-presents-report-new-genetic-engineering

by independent scientists, who were not involved in its compilation and who are free of any interests in the application of the technology.

References

Adikusuma F, Piltz S, Corbett MA, Turvey M, McColl SR, Helbig KJ, Beard MR, Hughes J, Pomerantz RT, Thomas PQ (2018) Large deletions induced by Cas9 cleavage. Nature 560(7717):E8-E9. doi:10.1038/s41586-018-0380-z

Belfield EJ, Ding ZJ, Jamieson FJC, Visscher AM, Zheng SJ, Mithani A, Harberd NP (2018) DNA mismatch repair preferentially protects genes from mutation. Genome Res 28(1):66-74. doi:10.1101/gr.219303.116

Biswas S, Tian J, Li R, Chen X, Luo Z, Chen M, Zhao X, Zhang D, Persson S, Yuan Z, Shi J (2020) Investigation of CRISPR/Cas9-induced SD1 rice mutants highlights the importance of molecular characterization in plant molecular breeding. J Genet Genomics 47(5):273-280. doi:10.1016/j.jgg.2020.04.004

Brinkman EK, Chen T, de Haas M, Holland HA, Akhtar W, van Steensel B (2018) Kinetics and fidelity of the repair of Cas9-induced double-strand DNA breaks. Mol Cell 70(5):801-813 e806. doi:10.1016/j.molcel.2018.04.016

Burgio G, Teboul L (2020) Anticipating and identifying collateral damage in genome editing. Trends in Genetics 36(12):905-914. doi:10.1016/j.tig.2020.09.011

Cho SW, Kim S, Kim Y, Kweon J, Kim HS, Bae S, Kim JS (2014) Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. Genome Res 24(1):132-141. doi:10.1101/gr.162339.113

Eckerstorfer MF, Dolezel M, Heissenberger A, Miklau M, Reichenbecher W, Steinbrecher RA, Wassmann F (2019) An EU perspective on biosafety considerations for plants developed by genome editing and other new genetic modification techniques (nGMs). Front Bioeng Biotechnol 7:31. https://doi.org/10.3389/fbioe.2019.00031

EFSA GMO Panel (2021) Scientific Opinion on the evaluation of existing guidelines for their adequacy for the molecular characterisation and environmental risk assessment of genetically modified plants obtained through synthetic biology. EFSA Journal 19(2):6301. https://doi.org/10.2903/j.efsa.2021.6301

Enfissi EMA, Drapal M, Perez-Fons L, Nogueira M, Berry HM, Almeida J, Fraser PD (2021) New Plant Breeding Techniques and their regulatory implications: An opportunity to advance metabolomics approaches. Journal of Plant Physiology:153378. doi:https://doi.org/10.1016/j.jplph.2021.153378

Forsbach A, Schubert D, Lechtenberg B, Gils M, Schmidt R (2003) A comprehensive characterization of single-copy T-DNA insertions in the Arabidopsis thaliana genome. Plant Mol Biol 52(1):161-176. doi:10.1023/a:1023929630687

Gelvin SB (2017) Integration of Agrobacterium T-DNA into the plant genome. Annu Rev Genet 51:195-217. doi:10.1146/annurev-genet-120215-035320

Grunewald J, Zhou R, Garcia SP, Iyer S, Lareau CA, Aryee MJ, Joung JK (2019) Transcriptome-wide off-target RNA editing induced by CRISPR-guided DNA base editors. Nature 569(7756):433-437. doi:10.1038/s41586-019-1161-z

Haapaniemi E, Botla S, Persson J, Schmierer B, Taipale J (2018) CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. Nat Med 24(7):927-930.. doi:10.1038/s41591-018-0049-z

Jupe F, Rivkin AC, Michael TP, Zander M, Motley ST, Sandoval JP, Slotkin RK, Chen H, Castanon R, Nery JR, Ecker JR (2019) The complex architecture and epigenomic impact of plant T-DNA insertions. PLoS Genet 15 (1):e1007819. doi:10.1371/journal.pgen.1007819

Kannan, B., Jung, J. H., Moxley, G. W., Lee, S. M., Altpeter, F. (2018) TALEN-mediated targeted mutagenesis of more than 100 COMT copies/alleles in highly polyploid sugarcane improves saccharification efficiency without compromising biomass yield. Plant Biotechnol J 16(4): 856-866. https://doi.org/10.1111/pbi.12833

Kapahnke M, Banning A, Tikkanen R (2016) Random splicing of several exons caused by a single base change in the target exon of CRISPR/Cas9 mediated gene knockout. Cells 5 (4). doi:10.3390/cells5040045

Kawall K (2019) New possibilities on the horizon: genome editing makes the whole genome accessible for changes. Front Plant Sci 10:525. doi:10.3389/fpls.2019.00525

Kawall K, Cotter J, Then C (2020) Broadening the EU GMO risk assessment in the EU for genome editing technologies in Agriculture. Environ Sci Eur 32(1):1-24. https://doi.org/10.1186/s12302-020-00361-2

Kawall K (2021) Genome edited Camelina sativa with a unique fatty acid content and its potential impact on ecosystems, Environ Sci Eur 33(1):1-12. https://doi.org/10.1186/s12302-021-00482-2

Kosicki M, Tomberg K, Bradley A (2018) Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. Nat Biotechnol 36(8):765-771. doi:10.1038/nbt.4192

Kosicki M, Allen F, Bradley A (2020) Cas9-induced large deletions and small indels are controlled in a convergent fashion. bioRxiv. doi:10.1101/2020.08.05.216739

Lalonde S, Stone OA, Lessard S, Lavertu A, Desjardins J, Beaudoin M, Rivas M, Stainier DYR, Lettre G (2017) Frameshift indels introduced by genome editing can lead to in-frame exon skipping. PLoS One 12(6):e0178700. doi:10.1371/journal.pone.0178700

Leibowitz ML, Papathanasiou S, Doerfler PA, Blaine LJ, Yao Y, Zhang C-Z, Weiss MJ, Pellman D (2020) Chromothripsis as an on-target consequence of CRISPR-Cas9 genome editing. bioRxiv . doi:10.1101/2020.07.13.200998

Liu M, Zhang W, Xin C, Yin J, Shang Y, Ai C, Li J, Meng F-l, Hu J (2021) Global detection of DNA repair outcomes induced by CRISPR-Cas9. bioRxiv:2021.2002.2015.431335. doi:10.1101/2021.02.15.431335

Makarevitch I, Svitashev SK, Somers DA (2003) Complete sequence analysis of transgene loci from plants transformed via microprojectile bombardment. Plant Mol Biol 52(2):421-432. doi:10.1023/a:1023968920830

Michno JM, Virdi K, Stec AO, Liu J, Wang X, Xiong Y, Stupar RM (2020) Integration, abundance, and transmission of mutations and transgenes in a series of CRISPR/Cas9 soybean lines. BMC Biotechnol 20(1):10. doi:10.1186/s12896-020-00604-3

Morineau C, Bellec Y, Tellier F, Gissot L, Kelemen Z, Nogue F, Faure JD (2017) Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid Camelina sativa. Plant Biotechnol J 15(6):729-739. doi:10.1111/pbi.12671

Monroe JG, Srikant T, Carbonell-Bejerano P, Exposito-Alonso M, Weng M-L, Rutter MT, Fenster CB, Weigel D (2020) Mutation bias shapes gene evolution in Arabidopsis thaliana. bioRxiv. doi:10.1101/2020.06.17.156752

Norris AL, Lee SS, Greenlees KJ, Tadesse DA, Miller MF, Lombardi HA (2020) Template plasmid integration in germline genome-edited cattle. Nat Biotechnol 38(2):163-164. doi:10.1038/s41587-019-0394-6

Ono R, Yasuhiko Y, Aisaki KI, Kitajima S, Kanno J, Hirabayashi Y (2019) Exosome-mediated horizontal gene transfer occurs in double-strand break repair during genome editing. Commun Biol 2:57. doi:10.1038/s42003-019-0300-2

Rang A, Linke B, Jansen B (2005) Detection of RNA variants transcribed from the transgene in Roundup Ready soybean. Eur Food Res Technol 220(3):438-443. doi:10.1007/s00217-004-1064-5

Ribarits A, Eckerstorfer M, Simon S, Stepanek W (2021) Genome-edited plants: opportunities and challenges for an anticipatory detection and identification framework. Foods 10(2):430. doi: 10.3390/foods10020430

Rostoks N (2021) Implications of the EFSA Scientific Opinion on Site Directed Nucleases 1 and 2 for Risk Assessment of Genome-Edited Plants in the EU. Agronomy 11(3):572. doi:10.3390/agronomy11030572

Sanchez-Leon S, Gil-Humanes J, Ozuna CV, Gimenez MJ, Sousa C, Voytas DF, Barro F (2018) Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. Plant Biotechnol J 16: 902-910. https://doi.org/10.1111/pbi.12837

Sharpe JJ, Cooper TA (2017) Unexpected consequences: exon skipping caused by CRISPR-generated mutations. Genome Biol 18(1):109. doi:10.1186/s13059-017-1240-0

Shen L, Hua Y, Fu Y, Li J, Liu Q, Jiao X, Xin G, Wang J, Wang X, Yan C, Wang K (2017) Rapid generation of genetic diversity by multiplex CRISPR/Cas9 genome editing in rice. Sci China Life Sci 60(5):506-515. doi:10.1007/s11427-017-9008-8

Skryabin BV, Kummerfeld D-M, Gubar L, Seeger B, Kaiser H, Stegemann A, Roth J, Meuth SG, Pavenstädt H, Sherwood J, Pap T, Wedlich-Söldner R, Sunderkötter C, Schwartz YB, Brosius J, Rozhdestvensky TS (2020) Pervasive head-to-tail insertions of DNA templates mask desired CRISPR-Cas9–mediated genome editing events. Science Advances 6(7):eaax2941. doi:10.1126/sciadv.aax2941

Testbiotech (2020) Why ‘New GE’ needs to be regulated, Frequently Asked Questions on ‘New Genetic Engineering’ and technical backgrounds for CRISPR & Co. https://www.testbiotech.org/node/2659

Tuladhar R, Yeu Y, Tyler Piazza J, Tan Z, Rene Clemenceau J, Wu X, Barrett Q, Herbert J, Mathews DH, Kim J, Hyun Hwang T, Lum L (2019) CRISPR-Cas9-based mutagenesis frequently provokes on-target mRNA misregulation. Nat Commun 10(1):4056. doi:10.1038/s41467-019-12028-5

Weisheit I, Kroeger JA, Malik R, Klimmt J, Crusius D, Dannert A, Dichgans M, Paquet D (2020) Detection of Deleterious On-Target Effects after HDR-Mediated CRISPR Editing. Cell Rep 31(8):107689. doi:10.1016/j.celrep.2020.107689

Windels P, De Buck S, Van Bockstaele E, De Loose M, Depicker A (2003) T-DNA integration in Arabidopsis chromosomes. Presence and origin of filler DNA sequences. Plant Physiol 133(4):2061-2068. doi:10.1104/pp.103.027532

Wolt JD, Wang K, Sashital D, Lawrence-Dill CJ (2016) Achieving plant CRISPR targeting that limits off-target effects. Plant Genome 9(3):plantgenome2016.05.0047. doi:10.3835/plantgenome2016.05.0047

Zsogon A, Cermak T, Naves ER, Notini MM, Edel KH, Weinl S, Freschi L, Voytas DF, Kudla J, Peres LEP (2018) De novo domestication of wild tomato using genome editing. Nat Biotechnol 36:1211-1216. doi:10.1038/nbt.4272