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Stakeholder questionnaire on new genomic techniques to contribute to a Commission study requested by the Council

Fields marked with * are mandatory.

Questionnaire on new genomic techniques to contribute to the study requested by the Council

Discussed and finalised in the Ad-hoc Stakeholder meeting on 10 February 2020

Background

The Council has requested [1] the Commission to submit, by 30 April 2021, "a study in light of the Court of Justice's judgment in Case C-528/16 regarding the status of novel genomic techniques under Union law" (*i.e.* Directive 2001/18/EC, Regulation (EC) 1829/2003, Regulation (EC) 1830/2003 and Directive 2009/41/EC).

To respond to this Council's request, the Commission is collecting contributions from the stakeholders through the questionnaire below. The study covers all new genomic techniques that have been developed after 2001.

Instructions

For the purpose of the study, the following definition for new genomic techniques (NGTs) is used: techniques that are capable of altering the genetic material of an organism and which have emerged or have been developed since 2001 [2].

Unless specified otherwise, the term "NGT-products" used in the questionnaire covers plants, animals, micro-organisms and derived food and feed products obtained by NGTs for agri-food, medicinal and industrial applications and for research.

Please substantiate your replies with explanations, data and source of information as well as with practical examples, whenever possible. If a reply to a specific question only applies to specific NGTs/organisms, please indicate this in the reply.

Please indicate which information should be treated as confidential in order to protect the commercial

interests of a natural or legal person. Personal data, if any, will be protected pursuant to Regulation (EU) 2018/1725 [3].

[1] Council Decision (EU) 2019/1904, OJ L 293 14.11.2019, p. 103-104, https://eur-lex.europa.eu/eli/dec/2019/1904/oj
[2] Examples of techniques include: 1) Genome editing techniques such as CRISPR, TALEN, Zinc-finger nucleases, mega nucleases techniques, prime editing etc. These techniques can lead to mutagenesis and some of them also to cisgenesis, intragenesis or transgenesis. 2) Mutagenesis techniques such as oligonucleotide directed mutagenesis (ODM). 3) Epigenetic techniques such RdDM. Conversely, techniques already in use prior to 2001, such as Agrobacterium mediated techniques or gene gun, are not considered NGTs.
[3] Regulation (EU) 2018/1725 of the European Parliament and of the Council of 23 October 2018 on the protection of natural persons with regard to the processing of personal data by the Union institutions, bodies, offices and agencies and on the free movement of such data, and repealing Regulation (EC) No 45/2001 and Decision No 1247/2002/EC, OJ L 295, 21.11.2018, p. 39–98

Guidelines

Please note that the survey accepts a maximum of 5000 characters (with spaces) per reply field. You might be able to type more than 5000 characters, but then the text will not be accepted when you submit the questionnaire. You will also receive a warning message in red colour below the affected field.

You have the option to upload supporting documentation in the end of each section. You can upload multiple files, up to the size of 1 MB. However, note that any uploaded document cannot substitute your replies, which must still be given in a complete manner within the reply fields allocated for each question.

You can share the link from the invitation email with another colleague if you want to split the filling-out process or contribute from different locations; however, remember that all contributions feed into the same single questionnaire.

You can save the draft questionnaire and edit it before the final submission.

You can find additional information and help here: https://ec.europa.eu/eusurvey/home/helpparticipants

Participants have until 15 May 2020 (close of business) to submit the questionnaire via EUsurvey.

QUESTIONNAIRE

Please provide the full name and acronym of the EU-level association that you are representing, as well as your Transparency Registry number (if you are registered)

If the name of the association is not in English, please provide an English translation in a parenthesis

European Network of Scientists for Social and Environmental Responsibility, ENSSER, , 500373220835-51

Please mention the sectors of activity/fields of interest of your association

Sectors of activity: bringing together independent scientific expertise to develop public-good knowledge for the critical assessment of existing and emerging technologies. Fields of interest: genetic modification, agriculture, energy, climate, environment, food safety, public health, pesticides, endocrine disrupting compounds, electromagnetic fields, epigenetics, developmental biology.

If applicable, please indicate which member associations (national or EU-level), or individual companies /other entities have contributed to this questionnaire

Not applicable

If applicable, indicate if all the replies refer to a specific technique or a specific organism

Not applicable

A - Implementation and enforcement of the GMO legislation with regard to new genomic techniques (NGTs)

* **1. Are your members developing, using, or planning to use NGTs/NGT-products?**

- (●) Yes
- ( ) No
- ( ) Not applicable

* Please provide details

We use NGTs and NGT-products for scientific research into their performance, effects and consequences. From overall reports such as [Doudna, J. (2019) CRISPR's unwanted anniversary. Science, 366, 777.], to specific examples such as development of the technique, [Woo, J.W. et al. (2015) DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoprotein], and the use of gene drives, which can have global repercussions [Wilkins, K.E. et al. (2018) Pest demography critically determines the viability of synthetic gene drives for population control. Math Biosci, 305, 160-169]; all these are examples of source materials used to evaluate possible applications of NGTs and the need and difficulties of aligning such applications with GMO legislation.
For instance, one of our members currently runs two projects in which CRISPR/Cas9 ribonucleoprotein complex is used to modify plant cells (protoplasts), while no external DNA is added to the system. One of these projects focuses on developing detection and identification methods for products of gene-edited organisms that might enter the food chain. The second is dedicated to investigating off-target and adverse effects of Cas9 systems in plant cell metabolism. Such effects are relevant for risk assessment and management strategies.

* **2. Have your members taken or planned to take measures to protect themselves from unintentional use of NGT-products?**

- (●) Yes
- ( ) No
- ( ) Not applicable

* Please provide details

One ENSSER member, for instance, has the disposal of BSL1 certified laboratories but has also established routines compatible with BSL2 and 3 (autoclave requirements and containment). Due to the legal limbo in some ENSSER partner countries, this member has requested Biosafety Certificates from the host university as if they were working with new GMOs.

* 2 bis. Have you encountered any challenges?

- (●) Yes
- ( ) No

* Please provide details

We have taken measures to protect ourselves from unintentional use of identifiable NGT products, but due to the incompleteness or absence of authorised detection methods and labeling for NGT products, we cannot know if we are exposed to NGT products we have not identified as such.

* **3. Are you aware of initiatives in your sector to develop, use, or of plans to use NGTs/NGT-products?**

- (●) Yes
- ( ) No
- ( ) Not applicable

* Please provide details

We follow GMO approvals and/or consulting letters in case of de-regulation. We follow all attempts at application and commercialisation of NGTs and NGT products and assess them for their effects on agriculture, public health, the environment and biodiversity. We also monitor attempts at de-regulation of NGTs, which we oppose: cf. our statement “Products of new genetic modification techniques should be strictly regulated as GMOs”, 2017, https://ensser.org/publications/ngmt-statement/.

* **4. Do you know of any initiatives in your sector to guard against unintentional use of NGT-products?**

- ( ) Yes
- (●) No
- ( ) Not applicable

* 4 bis. Are you aware of any challenges encountered?

- (●) Yes
- ( ) No

* Please provide details

We observe a clear tendency (outside of our own circle) to use NGTs and to develop NGT products without guarding against unintentional use, which we think is irresponsible and dangerous in various respects (cf. answers to other questions below). There is immense pressure from interested companies, mostly by lobbying and publication of misleading data, to escape from regulation, thus allowing unintentional use to go unnoticed as well. There is a concerted attempt to undermine regulation, including labeling and traceability that would enable the instant recognition of NGTs, NGT-organisms and NGT products, which is necessary to detect unintentional use. There are attempts in various countries by proponents and specific interest groups to change regulation in order to obtain de-regulation or exemption for SDN1 and SDN2 to no longer fall under the GMO regulations. The documents produced to demand or justify such unwarranted changes are commonly a misrepresentation of facts, of what is known, of what is not known, of what is assumed and of what is hoped for. We experience an unprecedented deviation from what solid and honest science would demand (see ENSSER's 2017 statement mentioned under question 3).

* **5. Are your members taking specific measures to comply with the GMO legislation as regards organisms obtained by NGTs?**

Please also see question 8 specifically on labelling

- (●) Yes
- ( ) No
- ( ) Not applicable

* Please describe the measures and their effectiveness including details on the required financial, human resources and technical expertise

Our members (insofar as they have confirmed this to us) take all legal and biosafety measures to comply with domestic and international GMO regulations; they only develop research work (no commercial value) and apply the required containment measures.

* What best practices can you share?

Please see our answers to all other questions.

* 5 bis. What challenges have you encountered?

See our replies to questions 2, 4bis, 6, 7 and others.

* **6. Has your organisation/your members been adequately supported by national and European authorities to conform to the legislation?**

- ( ) Yes
- (●) No
- ( ) Not applicable

* What challenges have you encountered?

We are not getting any support from authorities in terms of training, revision, advice, etc. on specific measures for new technologies. Examples of the challenges this raises are given in our answers to questions 2 and 7.

* **7. Does your sector have experience or knowledge on traceability strategies, which could be used for tracing NGT-products?**

- (●) Yes
- ( ) No
- ( ) Not applicable

* Please describe the traceability strategy, including details on the required financial, human resources and technical expertise

We have established routine protocols for GMO detection and we have developed several research projects on detection. Our members are also part of the Online Network of Laboratories for the Detection and Identification of Living Modified Organisms (http://bch.cbd.int/onlineconferences/portal_detection /2019discussions.shtml) under the Cartagena Protocol of the Convention on Biological Diversity. Therefore, members are fully updated with new methods and challenges for NGT detection.
We have – for example – approved a large detection and identification project called “FOODPRINT: Traceability and labelling of gene-edited products in the food chain” with the Research Council of Norway (12 million NOK; 4 years-project; start date June 2020). This project will generate knowledge that will offer technically and economically feasible options for multiple stakeholders, such as industry, civil society organizations and regulators, on traceability and labelling of gene-edited products through a Responsible Research and Innovation (RRI) framework, and with potential impact for Norway and all EU member States as well as their trade partner countries. The project comprehends the active participation of stakeholders from academia, industry and regulatory agencies in Europe and Brazil. Therefore, it includes aspects of the entire food chain, from exporter to importer territories, and suppliers from GM and non-GM food chains. FOODPRINT goes beyond the idea that GMO detection has to be based on a single “transgenic tag”. The project advances from ‘single-target DNA approach’ to a ‘multi-target approach’ based on other layers of genetic information. The main goal of FOODPRINT is to develop, test and showcase – as a proof-of-concept – an innovative multi-flexible model for decision analysis on the detection and identification of a variety of GM products, including future gene-edited products, entering the Norwegian and European food market. The suitability of this model relies on the combination of traditional PCR detection methods and new methods that apply cutting edge techniques (i.e. omics), representing a significant advantage over current PCR-based matrix schemes. The use of sophisticated molecular profiling techniques will enable the identification of gene-editing events/individuals from unique patterns of multiple genetic signatures and marks generated without prior knowledge of the material. Hence, the approach is also suitable for the identification of unauthorized GMO occurrence. We focus not only on evaluating and applying the novel multi-target approach but also on implementing it in an easy-to-use way to motivate its operation under the EU system. In order to also address regulatory compliance and feasibility issues related to the currently used detection platforms, we will investigate reproducibility and harmonization approaches for enhancing underlying detection, identification and quantification methods. To empirically evaluate our work and to produce outputs of high societal relevance we will consider the active participation of stakeholders from industry, regulatory agencies and civil society organizations in Norway and other European countries.

* **8. Are your members taking specific measures for NGT-products to ensure the compliance with the labelling requirements of the GMO legislation?**

- (●) Yes
- ( ) No
- ( ) Not applicable

* Please describe the measures and their effectiveness including details on the required financial, human resources and technical expertise

Please see answer to question 7.

* What best practices can you share?

Please see answer to question 7.

* 8 bis. What challenges have you encountered?

Please see answer to question 7.

* **9. Do you have other experience or knowledge that you can share on the application of the GMO legislation, including experimental releases (such as field trials or clinical trials), concerning NGTs/NGT-products ?**

- (●) Yes
- ( ) No
- ( ) Not applicable

* Please describe for the:

- [x] Agri-food sector
- [x] Industrial sector
- [ ] Medicinal sector

Agri-food sector

We evaluate the principles as described under questions 15, 17 and 29.

Industrial sector

This does not refer specifically to the industrial sector, but there is no other space to give this reply. It refers to all sectors.
We shall also be happy to share knowledge on the potential misuse and abuse of NGTs, which is likely to happen and constitutes a serious consequence of these techniques. ENSSER has pointed out this risk in our 2017 statement “Products of new genetic modification techniques should be strictly regulated as GMOs” (https://ensser.org/publications/ngmt-statement/). Inadvertent misuse may for instance arise because “garage scientists” or biohackers can now obtain genome editing kits on the internet and produce their own genome-edited products. This is already happening. Further, the GMO legislation does not consider yet that NGTs may turn plants, food or feed products or other products into attack vectors for bioterrorism or biocrime. Just one genetic modification can transform a harmless bacterium into a pathogenic or antibiotic-resistant bacterium. This and other applications of genome editing techniques have become so easy to realise, that they open up the possibility of both intentional abuse and inadvertent misuse with an alarming likelihood. Much NGT research is “dual use research of concern” (DURC). NGTs are especially susceptible to underappreciated risks fostered by their cyber overlaps. This is explained in the two following articles:
S. Mueller: "On DNA Signatures, Their Dual-Use Potential for GMO Counterfeiting, and a Cyber-Based Security Solution", Front. Bioeng. Biotechnol., 07 August 2019, https://www.frontiersin.org/articles/10.3389/fbioe.2019.00189/full
S. Mueller: "Are Market GM Plants an Unrecognized Platform for Bioterrorism and Biocrime?", Front. Bioeng. Biotechnol., 29 May 2019, https://www.frontiersin.org/articles/10.3389/fbioe.2019.00121/full

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B - Information on research on NGTs/NGT-products

*** 10. Are your members carrying out NGT-related research in your sector?**

- (•) Yes
- ( ) No
- ( ) Not applicable

* Please specify including subject, type of research, resources allocated, research location

Examples:

- SynPlast (internal funds; approx. 17,000 euro annually) – investigation of off-target and adverse effects of CRISPR/Cas9 systems in plant cells. Research performed in Norway and collaborators in Brazil.
- FOODPRINT (funds from Research Council of Norway; approx. 1.066.000 euro for 4 years) – development of detection and identification methodologies for gene-edited products in the food chain. Research performed in Norway and collaborators in Brazil, Switzerland, Austria, France, Germany and Lebanon.
- Horizon Scanning Biotechnology performed by the “Fachstelle Gentechnik und Umwelt”, which started in march 2020 (funded by German Federal Agency for Nature Conservation (Bundesamt für Naturschutz, BfN): continuous horizon scanning process on new developments in the field of genome editing.
- Investigating the development, applications and risks of synthetic gene drives (including CRISPR/Cas-based gene drives), as well as their social aspects, ethics and regulation: https://genedrives.ch/. A two year project carried out by ENSSER, Critical Scientists Switzerland and Federation of German Scientists (Vereinigung Deutscher Wissenschaftler). The results were presented at a symposium in May 2019 and are also published as a book/report (CSS-ENSSER-VDW 2019) available at https://genedrives.ch/report/. The work was funded by the Mercator Foundation Switzerland. The work is continuing in international collaboration.

*** 11. Are you aware of other NGT-related research in your sector?**

- (•) Yes
- ( ) No
- ( ) Not applicable

* Please specify

For instance, genome editing research is being carried out in the field of human gene therapy, including safety and reliability research.

*** 12. Has there been any immediate impact on NGT-related research in your sector following the Court of Justice of the EU ruling on mutagenesis?**

Court of Justice ruling: Case C-528/16 http://curia.europa.eu/juris/documents.jsf?num=C-528/16

- (•) Yes

- ○ No
- ○ Not applicable

* Please describe

Although we had been doing research on e.g. detection of NGT products for years before the ruling, the ruling confirmed that this falls under GMO regulation, which made us expand our focus towards adaptation and implementation of GMO regulation for NGT organisms and products. For instance, we now develop new risk assessment approaches, to deal with the biological differences between classical GMOs and NGT organisms. Lack of appropriate and validated detection methods has a huge impact on risk assessment and monitoring. Also, risk hypothesis related to unintended effects needs to be revised. For instance, searching for sequences similar to the targeted ones in the host genome should be considered now, as well as whole genome sequencing for the molecular characterization step in risk assessment.

*** 13. Could NGT-related research bring benefits/opportunities to your sector/field of interest?**

- ◉ Yes
- ○ No
- ○ Not applicable

* Please provide concrete examples/data

For instance, gene regulation studies would benefit from NGT technologies.

*** 14. Is NGT-related research facing challenges in your sector/field of interest?**

- ◉ Yes
- ○ No
- ○ Not applicable

* Please provide concrete examples/data

Please see attachment.

*** 15. Have you identified any NGT-related research needs/gaps?**

- ◉ Yes
- ○ No
- ○ Not applicable

* Please specify which needs/gaps, explain the reasoning and how these needs/gaps could be addressed

We have identified many NGT-related research needs/gaps. These can be approached from different angles. An overview of these angles is given here and detailed considerations from several angles are given in the attachment.

Without claiming to be complete, one might summarise the most important NGT-related research needs and gaps as follows:

1) Within basic genetics: i) the pleiotropic actions of genes; whether and how this changes after editing; ii) the pleiotropic impacts of gene products, e.g. how the proteome is changed by gene editing other than the

targeted protein; iii) whether related control systems such as by non-coding RNA and methylation, are influenced by editing. All these indicate that NGTs are being applied in considerable ignorance of genetic properties that are relevant.
2) Impacts on agricultural intensification. i) If the NGT is successful in its immediate purpose, how does this impact on farming methods, and ii) on biodiversity? These are questions beyond immediate matters of safety.
3) Potential danger points; NGT techniques are powerful, and can be used for harm as well as good, as with poisons or guns. As the technique becomes simpler and more widespread, so it enables anyone to apply it easily. Therefore the potential misuse such as for war and terrorism, requires closer evaluation, leading probably to regulation much as for weapons that goes beyond that for GMOs. (Cf. answers to questions 9 and 14 sub b.)
4) The extended use of NGTs for gene drives poses both powerful advances and extreme hazards, as mentioned above, under questions 1 and 10. Research such as Wilkins et al 2018, quoted under question 1, examines how to model the process and its possible dangers. Research needs and gaps concerning gene drives can be found in the conclusions and recommendations mentioned in the summary of the report of the Gene Drive project (https://genedrives.ch/report/) mentioned in question 10.

As NGTs not only include so-called genome editing tools such as ZFNs or CRISPR/Cas, but many other novel genetic modification techniques as well, there is a whole range of research that would need to be undertaken to understand the risks and potential consequences better for each of them. Furthermore, they also offer tools for more general learning about the various processes and interactions in organisms (plants, animals, funghi), including communication within the cells, between cells, within organisms and between the organism and the external environment and its components, including intraspecies and interspecies communication and reactions. This is in particular true for epigenetics, where core knowledge is still lacking, including what exactly triggers an epigenetic response (such as sequence methylation), how long it will last and why (e.g. how many generations, or to which stage of development), what are the signals initiating or necessary for removing gene silencing and what are the differences between kingdoms, families or species. The field of molecular communication and transport within an organism is also crucial for fields such as transgrafting with either GM rootstock or GM graft, where knowledge is rudimentary. A NGT hat deserves much more attention for its consequences is RNA interference (RNAi), using double-stranded RNA (dsRNA, also called small interfering RNA or siRNA): see the work of Jack Heinemann (e.g. J. Heinemann and S. Walker, Environmentally applied nucleic acids and proteins for purposes of engineering changes to genes and other genetic material, Biosafety and Health 1, 3, 113-123 (2019), https://doi.org/10.1016/j.bsheal. 2019.09.003).

In all cases, i.e. for all types of NGTs, the knowledge needs to be obtained as to which unintended effects may occur and case-specifically do occur, and what the consequences are in the short as well as long term, both for the organism and for the impacts on the environment (incl. molecular communication, change of composition of surrounding organisms (e.g. in soil), attracting, repelling and responding to pests and pathogens, etc.). A good impression of NGT research needs and gaps is given by Eckerstorfer et al. 2019 [Eckerstorfer MF et al. (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. doi: 10.3389/fbioe.2019.00031].

NGT research needs and gaps can also be found in the objectives of the two research projects SynPlast and FOODPRINT mentioned in question 10: they investigate risk hypothesis (adverse effects) and legal implementation challenges (detection) in NGT plants and plant products.

More detailed considerations from several of the above angles are given in the attachment.

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

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C - Information on potential opportunities and benefits of NGTs/NGT-products

* **16. Could NGTs/NGT-products bring benefits/opportunities to your sector/field of interest?**

- (●) Yes
- ( ) No

* Please describe and provide concrete examples/data

For research, yes. For commercial purposes it needs to be addressed in a case-by-case technology assessment, that will also have to include additional parameters such as need, communal good, contribution to sustainability and resilience.

NGTs, in particular site-directed nucleases like CRISPR/Cas, but also transgrafting, RdDM, RNAi, can provide valuable research tools for many fields of inquiry. It would be a waste and misconception to view them primarily as technologies for direct product development resulting in new GMOs.

* Are these benefits/opportunities specific to NGTs/NGT-products?

- (●) Yes
- ( ) No

* Please explain

See first explanation to this question.

* **17. Could NGTs/NGT-products bring benefits/opportunities to society in general such as for the environment, human, animal and plant health, consumers, animal welfare, as well as social and economic benefits?**

- (●) Yes
- ( ) No

* Please describe and provide concrete examples/data

The research we referred to in the previous question (and in other questions) will raise our knowledge in many areas and thereby benefit society (cf. our answer to question 16). Whether society will benefit from NGT products as such is a question. In this respect, the “could” in this question is problematic, as anything could be listed here as a benefit or opportunity without it requiring a solid benefit analysis, and without having a societal agreement of what constitutes a benefit and what does not. Also, what about a benefit for one that is a hardship for others? This is a major limitation of the current EU GMO legislation: risks are assessed, but benefits are not.

The knowledge of how to ‘edit’ the genome (the term is not fitting) or modify it, would seem to present untold opportunities. Yet these can be realised more surely with established methods especially using new insights such as from systems biology, into how the biosphere operates. It is our judgement that these will provide what is needed for agriculture and related areas, towards a more resilient future and some approach towards the UN Sustainable Development Goals.

When used as a research tool and with the aim for better understanding for example the parameters of resilience and sustainability, or the role of specific genes or regulatory sequences in medical context, then NGTs can be a valuable contributor amongst other methods and technologies.

Without a deeper, systematic, inter- and intradisciplinary deliberation and analysis of underlying causes to perceived problems (often symptoms) there will be a tendency to resort to quick technological answers (sometimes referred to as techno-fixes) – which at the time may be perceived as a benefit. Such a short-term and technology-centred rather than solution-centred approach has the capacity to further deepen problems or create new ones in the long run. Hence without a well-thought-through framework as to what constitutes a benefit and how to assess this, devised under broad public participation, the question for benefits as provided here is of little help.

* Under which conditions do you consider this would be the case?

See our first explanation under this question.

* Are these benefits/opportunities specific to NGTs/NGT-products?

- (●) Yes
- ( ) No

* Please explain

See our first explanation under this question.

* **18. Do you see particular opportunities for SMEs/small scale operators to access markets with their NGTs/NGT-products?**

- ( ) Yes
- (●) No

* Please explain why not

There is no “particular” opportunity here for accessing markets. There may be a dream of this, but the reality of what can factually be done with these technologies and whether the outcome is reliable, whether it is wanted by the market and the consumer and is not adding to further problem creation – all this has many question marks. Access to market is not a simple opportunity arising from access to the technology.

* **19. Do you see benefits/opportunities from patenting or accessing patented NGTs/NGT-products?**

- ○ Yes
- ◉ No

* Please explain why not

There is always benefit for those who own the patent, but this is not always a benefit for society. In particular when the food chain and biodiversity are concerned (which can be properly treated as human rights), the patenting process is inadequate: not enough security/safety tests, not enough independent reviews, biased results, etc. See also our answer to question 17.

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D - Information on potential challenges and concerns on NGTs/NGT-products

* **20. Could NGTs/NGT-products raise challenges/concerns for your sector/field of interest?**

- ◉ Yes
- ○ No

* Please describe and provide concrete examples/data

See attachment 2.

* Are these challenges/concerns specific to NGTs/NGT-products?

- ◉ Yes
- ○ No

* Please explain

See attachment 2.

* **21. Could NGTs/NGT-products raise challenges/concerns for society in general such as for the environment, human, animal and plant health, consumers, animal welfare, as well as social and economic challenges?**

- ◉ Yes
- ○ No

* Please describe and provide concrete examples/data

Without an overall and public participatory technology assessment, without a clear understanding and concept of how for example agriculture can be part of the solution rather than part of the problem (climate change, biodiversity loss, collapse of ecosystems, their function and services) and without taking clear guidance from the precautionary principle, an enhanced use and application of NGTs in the farming or forestry sector is likely to exacerbate problems. If underlying causes are not taken care of by appropriate action, practices and technologies, problems will continue to arise and deepen. The reasons for this have been stated in our answers to the above questions, particularly 14, 15 and 20.
Whilst current guidance and practice for case by case risk assessment are already challenged by some of the NGTs, case by case risk assessment is insufficient for revealing the bigger picture and attending to the bigger questions that NGTs often claim to be an answer to.

A concrete example of a concern come true is the affair of the genome-edited hornless cattle. A company from the USA bred cattle which it had made hornless by germline modification using the NGT TALENS (D.F. Carlson et al. Production of hornless dairy cattle from genome-edited cell lines. Nature Biotechnology 34, 479–481 (2016), https://www.nature.com/articles/nbt.3560#article-info). It claimed that the animals were “free of off-target events”, but when the FDA analysed whole genome sequencing data from the cattle, it discovered the unintended presence of bacterial DNA, including bacterial antibiotic resistance genes, in the cattle genome (A.L. Norris et al. Template plasmid integration in germline genome-edited cattle. Nature Biotechnology volume 38, pages 163–164 (2020), https://www.nature.com/articles/s41587-019-0394-6_). The bacterial DNA came from the bacterial plasmid (a DNA carrier) used to introduce the DNA for the hornless trait into the cattle. This constitutes a risk, as bacteria could take over the antibiotic resistance genes from the cattle and thus contribute to the large medical and veterinary problem of antibiotic resistance. Another concern was that the whole genome sequencing data were actually present in the 2016 paper, but the company had not cared to analyse them for unintended effects, despite their claim to the contrary. The FDA therefore argues for regulation of NGTs in animals (https://www.fda.gov/news-events/press-announcements/fda-expertise-advancing-understanding-intentional-genomic-alterations-animals).

This is an example of the tendency to resort to techno-fixes rather than solve the root cause of a problem. Many proposed applications of NGTs are prime examples of techno-fixes.

Apart from such unintended consequences, NGTs also have a large potential for active abuse, as we explained in questions 9 and 14 sub b. The possibilities to develop biological weapons, for instance, have been greatly expanded by the technical power of NGTs.

* Under which conditions do you consider this would be the case?

See our first explanation under this question.

* Are these challenges/concerns specific to NGTs/products obtained by NGTs?

- (●) Yes
- ( ) No

* Please explain

See our first explanation under this question.

*

22. Do you see particular challenges for SMEs/small scale operators to access markets with their NGTs /NGT-products?

- (●) Yes
- ( ) No

* Please explain and provide concrete examples and data

a) If patents are granted to large multinational companies, small scale operators will have to pay royalties to the large companies, as they have also done with classic GMO seeds. Also, smaller SMEs are less likely to be able to afford proper safety studies. The main problem for SMEs is not NGT regulation, but the costs of research and patents: due to these, SMEs can only be successful with NGTs if they are taken over by large companies, as ENSSER member Michael Antoniou recently argued (N. Foote, Euractiv.com: “Gene-editing regulation not the biggest hurdle for SMEs in EU, says academic”, 27 Feb. 2020, https://www.euractiv.com /section/agriculture-food/news/gene-editing-regulation-not-the-biggest-hurdle-for-smes-in-eu-says-academic). Thus, NGTs contribute to the concentration of markets in the hands of multinational companies.

b) Deregulation of NGTs, or any arrangement allowing NGTs to be used in agriculture without supervision, labeling and traceability, will make organic agriculture essentially impossible. NGTs are genetic modification techniques and organic agriculture does not allow the use of genetic modification. Thus the existence of the whole organic sector, consisting mainly of farmers and SMEs, is threatened by downregulation of NGTs because this will make NGT products unrecognisable as GMO products.

*** 23. Do you see challenges/concerns from patenting or accessing patented NGTs/NGT-products?**

- (●) Yes
- ( ) No

* Please describe and provide concrete examples/data

a) Please see our reply to question 22.

b) Due to patents, it is almost impossible to access NGTs and their products for independent safety assessments, e.g. for animal safety studies to look for toxic outcomes. Due to patents, a researcher can’t just buy materials to test. For example, it is the experience of one of our members that trying to get patented GM crops to test from a seed merchant meant that the buyer needed to sign a legal user/stewardship agreement stipulating that the seeds could not be used for research, nor could they be given to anyone else for research, either. As a result, if the researcher had conducted safety assessments on the material, he/she could have been sued. Instead, researchers were required to apply directly to the patent-holder for materials to test. In the experience of our member, this delayed their independent safety studies for years. In the end, test materials were obtained from a farmer who had grown the GM crop, where the farmer was not told that the crop was to be fed to research animals, in order to protect the farmer from possible legal action. In contrast, the patent-holder and their approved researchers have little trouble obtaining materials to test, resulting in a possible bias in the safety findings of published studies.

c) Another concern of ours is that patents could themselves become Trojan horses to enable a new form of DURC (dual use research of concern) that has not been recognized. Patents are often weak, premature, and possibly exploitable for abuse. Especially in the context of NGTs, we are aware of numerous patented technologies that rely on physical interactions that have never been assessed for their potential to cause harm, either by accident or intentionally (see J. Heinemann and S. Walker, Environmentally applied nucleic

acids and proteins for purposes of engineering changes to genes and other genetic material, Biosafety and Health 1, 3, 113-123 (2019), https://doi.org/10.1016/j.bsheal.2019.09.003). We need a more rigorous and open discussion to assess any possible concerns of NGTs related to patents.

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E - Safety of NGTs/NGT-products

*** 24. What is your view on the safety of NGTs/NGT-products? Please substantiate your reply**

See attachment 3

*** 25. Do you have specific safety considerations on NGTs/NGT-products?**

- (●) Yes
- ( ) No

* Please explain

- Genome-editing might not result in only a single reproducible mutation; it can be many mutations, in many organisms, at the same time.
- Genome-editing mutations are different from spontaneous “natural” mutations because they function on sequence similarity basis and because of differences in the repair mechanism.
- Not all target and off-target sites are edited in a predictable manner.
- Risk hypothesis should not be restricted to the presence or absence of foreign DNA or RNA.
- Untargeted screening approaches are needed to search for unintended changes in the genotype and phenotype.
- Risk hypotheses are not mutually exclusive.
- Biosafety implications do not correlate with a level-based regulation.
- The Cartagena Protocol has a valid framework for risk assessment.
- Analytical gaps, such as detection and scalability, are foreseen for genome-editing and RNAi (RNA interference) products.
- New technologies generate new uncertainties and new risk hypotheses.
- There is a need to perform and communicate uncertainty analysis to make informed decisions.
- RRI (Responsible Research and Innovation) may provide a way to perform a technology assessment at its development stage (public funded).

Safety concerns about NGTs and NGT products have not been adequately and fully assessed. Many of the current testing methods are inappropriate. We need more rigorous and independent tests to demonstrate safety. The tests and methods and their interpretations need to be independently assessed, also by experts in other fields, e.g. statistics. A recent paper highlights the serious concern of how unreliable most (!) statistical tests have become in the applied sciences: Szucs D and Ioannidis JPA (2017): "When Null Hypothesis Significance Testing Is Unsuitable for Research: A Reassessment", Front. Hum. Neurosci. 11: 390, doi: 10.3389/fnhum.2017.00390.

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F - Ethical aspects of NGTs/NGT-products

*** 26. What is your view on ethical aspects related to NGTs/NGT-products? Please substantiate your reply**

See attachment 4

*** 27. Do you have specific ethical considerations on NGTs/NGT-products?**

- (●) Yes
- ( ) No

* Please explain

a) NGT applications are proposed for use in the conservation of biodiversity (e.g. interventions in natural populations of corals and trees), which raises serious ethical and safety issues.

b) NGTs, especially CRISPR/Cas, are being used in the design of synthetic gene drives and gene drive organisms (GDOs). As they are designed to genetically modify, replace or eradicate populations or species in the wild, there are serious ethical issues involved. This aspect though is not a case for decisions by ethic committees, but rather needs a broad and participatory public debate as well as technology assessment. The unique and strong hazards and risks of synthetic gene drives add equally to the ethical dimension. See for example Simon, S. et al. (2018) Synthetic gene drive: between continuity and novelty: Crucial differences between gene drive and genetically modified organisms require an adapted risk assessment for their use. EMBO Rep 19 (5), https://doi.org/10.15252/embr.201845760.

c) NGTs enable human beings to deeply intervene into living organisms and systems and to manipulate nature to a new, unprecedented extent. Furthermore, if released into the environment, NGT-products can have irreversible consequences for the whole ecosystem. It is undeniable that these are deeply ethical issues.

A precautionary situation is one in which harm could occur, but where there is only limited knowledge about the probability of the occurrence of this possible harm. The precautionary principle is a response to such situations of uncertainty. The ethical idea of precaution justifies an obligation to take measures to prevent possible serious harm or, if harm does occur, to limit it to an extent not exceeding a permissible degree. This obligation exists even if no more is (yet) known about the probability of occurrence other than that it is above zero – and neither a qualitative nor a quantitative probability can be defined.

Precautionary measures serve to shape the situation, e.g. with regard to new technologies, so as to minimise the probability of serious harm occurring, while enabling the collection of the necessary data to acquire the knowledge needed to assess the level of the risk. As long as we are in the area of precaution, it is not a matter of ‘weighing up’ or ‘balancing’ risks and opportunities, but of identifying the unknown probabilities of potential serious harm. Only once these risks are known, can they be evaluated.

The precautionary principle neither inhibits nor is hostile to innovation. While it does stress the potential for serious harm, it also demands a broadening of knowledge about opportunities and encourages alternative development paths, which may entail less potential harm but equal (or greater) potential benefits, to be considered at an early stage of product development.

In addition to this, we are highly concerned about the potentially devastating consequences of the use of NGT-products in both gene drives and biological warfare.

References:
ECNH/Federal Ethics Committee on Non-Human Biotechnology 2018: Precaution in the environmental field. https://www.ekah.admin.ch/inhalte/ekah-dateien/dokumentation/veranstaltungen/Veranstaltung_7._Mai_2018/EKAH_Broschu__re_Vorsorge_Umweltbereich_e__18_Web_V2.pdf

ECNH/Federal Ethics Committee on Non-Human Biotechnology 2019a: Gene Drives. Ethical Considerations on the use of Gene Drives in the Environment. https://www.ekah.admin.ch/inhalte/ekah-dateien/dokumentation/publikationen/EKAH_Bericht_Gene_Drives_EN_V2.pdf

ECNH/Federal Ethics Committee on Non-Human Biotechnology 2019b: Does the precautionary principle needs to be supplemented? Ethical Considerations on the “innovation principle”. https://www.ekah.admin.ch/inhalte/ekah-dateien/dokumentation/publikationen/EKAH_Innovationsbericht_EN_V.pdf

Willemsen, A., Rippe, K. P. 2018: The Idea of Precaution: Ethical Requirements for the Regulation of New Biotechnologies in the Environmental Field. In: Frontiers in Plant Science, December 2018 | Volume 9 | Article 1868 doi: 10.3389/fpls.2018.01868

Wynne, B. 2019: Ethical Questions Raised by Gene-editing GM Technologies – and by their Modes of Governance. Presentation at European Commission’s Ethics Expert Group Roundtable on Gene-Editing on 16 October 2019. https://ensser.org/from-our-members/ethical-questions-raised-by-gene-editing-gm-technologies-and-by-their-modes-of-governance/

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G - Consumers' right for information/freedom of choice

*** 28. What is your view on the labelling of NGT-products? Please substantiate your reply**

NGTs pose challenges for consumers due to the lack of methods for detecting and identifying some types of gene-edited products using current GMO detection approaches. Detection and identification methods need to be developed in order to ensure consumers’ rights and GMO labelling legal requirements. Please see answers above, particularly about the research project FOODPRINT.

Labelling should be mandatory and measures should be taken to protect conventional production of seeds, food and feed, livestock, trees, etc. in order to enable freedom of choice for breeders, farmers, foresters and consumers. Current EU legislation provides for freedom of choice.

Labelling is also quintessential for the ability to understand and trace the source of a problem, should for

example allergies or other food-related problems arise. Once an NGT organism has been released into the environment or food supply, determining whether it or its products cause illness in the community relies on epidemiological work that tries to find a link between exposure to an NGT organism and a particular disease. Such studies cannot be done if there is no ability to measure exposure. For food-related exposures, exposure can only be measured by asking the ill person what he/she has been eating. Determining whether any of those foods involved exposure to a NGT organism can only be determined by looking on the label or asking the food manufacturer. If NGT products are not labelled, none of the researchers, consumers, or manufacturers will know if a particular foodstuff contains a particular NGT product. Therefore, if NGT products are not labelled, there will be almost no ability to determine if the NGT product has caused illness in the community. Even if there are many thousands of cases of ill people, there will be almost no ability to recall the NGT product and hence almost no ability to prevent further cases of illness. For public health reasons, it is therefore vital to label products containing NGTs.

In light of security, labelling of NGT products is an absolute must: see the two papers by S. Mueller mentioned under question 9 and the nascent cyberbiosecurity paradigm. Without compulsory labelling, abuse and misuse of NGTs are given free reign.

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H - Final question

*** 29. Do you have other comments you would like to make?**

- (●) Yes
- ( ) No

Please provide your comments here

See attachment 5

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Contact

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Attachment 1 to questionnaire on NGTs
ENSSER

14. Is NGT- related research facing challenges in your sector/field of interest? *Yes/no/not applicable*

- If yes, please provide concrete examples/data.
- If no, please explain why not.

a) The legal limbo among countries. This puts researchers in a vulnerable position due to the potential lack of regulation and compulsory biosafety measures.

b) Numerous security aspects are under-appreciated and have received little attention. It is imperative that dangers, concerns or risks in this area be rigorously and independently analysed. Consider the entirely new field of cyber-bio-security. The concern that this new discipline is describing, is that NGTs are susceptible to new forms of social engineering attacks; more generally, purported solutions could easily be subverted for sinister purposes. This situation is different from that in classical cyber-security. There is a critical gap between the product or entity in question (e.g. a specific GMO) and its digital description. This gap is important as it could lead to unintended errors (at best), or even be actively misused by those intending to do harm. GMOs are not tamper-proof and at the same time they are difficult to authenticate (see the two publications by S. Mueller quoted in question 9). A recent GMO feed contamination event in Europe could have been the result of this (largely unrecognized) concern.

Another new potential risk is that physical entities (materials, chemicals) – at a scale that cannot be verified and inspected with the naked eye or a traditional mechanical test – may be switched so that the actual intended product, entity or pathway does not match its digital (intended) description. This can make it difficult to identify which is which. It is rather easy, for instance, to swap two plasmids intentionally or unintentionally, or to introduce some changes in parts of the genome. The cyber overlap may also lead to manipulation of equipment and tests, disrupting quality control, etc.

Xenogenic escapes, in terms of microorganisms from high-security labs, should be taken much more seriously. So should the interactions between engineered and natural organisms: what do we know about these, when we are not even aware of all the players that are, or can be, involved? One of the dilemmas of investigating this is that real-life situations cannot be created in laboratory settings.

NGTs and their products are particularly vulnerable to cyberbiosecurity issues because of their high camouflaging opportunity (intended abuse), but also in the context of unintended effects. Their potential for both intended abuse and unintended misuse (fostered by the cyber overlaps and the unrecognized safety gaps this is creating) have not been fully assessed. The field of cyberbiosecurity is gaining a little more intention now: see the recent Frontiers Research Topic on Cyberbiosecurity (https://www.frontiersin.org/research-topics/8353/mapping-the-cyberbiosecurity-enterprise).

c) There is a lack of detailed data in most research publications regarding genome-wide off-target effects of genome editing tools. The lack of systematic acquisition and evaluation of whole genome sequencing data prevents proper assessment and predictions. Furthermore, the limitation of sequence evaluation to potential off-target sites predicted according to algorithms leads to false certainties and assumptions. Most genome editing studies use biased methods (conventional PCR) to screen the genome at predicted sites for off-target effects and only a minor portion of studies are using unbiased methods (whole genome sequencing) (Modrzejewski , D., Hartung, F., Sprink, T., Krause, D., Kohl, C., Wilhelm R. (2019) What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a systematic map. . Environ Evid.).

Whilst there is an increasing reliance on the use of algorithms to calculate and predict the potential off-target sites, according to the degree of homology (based on the number and position of mismatches), there is also increasing concern about this. In fact, the sole reliance on algorithms to accurately predict the potential off-target sites or regions for off- or on-target effects has come into question repeatedly, as only whole genome sequencing, an increasingly affordable technology, would be able to pick up some of the mutational effects observed. This does not only refer to extensive mutations delinked from the actual cutting site (Kosicki et al. 2018, *Nature Biotechnology* 36 (8):765-+. doi: 10.1038/nbt.4192.), but also to the integration of vector backbone DNA derived from the plasmid used in the original transgene construct, and for example observed in genome editing experiments with oilseed rape (Braatz et al. 2017, *Plant Physiology* 174 (2):935-942. doi: 10.1104/pp.17.00426).

Akcakaya et al. (Akcakaya et al. 2018, *Nature* 561 (7723):416-+. doi: 10.1038/s41586-018-0500-9) find that many studies reporting no or few off-target effects (mutations) will have failed to identify actual off-target effects due to the limitations of the "*in silico*" (i.e. computer modelling) predictions of potential off-target sites.

Lack of data and prevalence of assumptions thus create challenges to risk research as well as to predictive research.

Furthermore, chromosomal rearrangements can be identified using long-range PCR or long-read next generation sequencing platforms, such as the Pacific BioSciences or Oxford Nanopore Technology. However, these techniques are rarely used for routine genotyping of CRISPR/Cas-induced mutations in plants, so these on-target effects are likely to have remained undetected in many studies (Hahn F, Nekrasov V (2019) CRISPR/Cas precision: do we need to worry about off-targeting in plants? Plant cell reports 38 (4):437-441. doi:10.1007/s00299-018-2355-9).

Question 15. Have you identified any NGT-related research needs/gaps? Yes/no/not applicable
- If yes, please specify which needs/gaps, explainthe reasoning and how the needs/gaps could be addressed.

NGT-related research needs and gaps considered in more detail:

a) Unintended effects.

There is a need for in-depth investigations of unintended effects, which
1.) occur during the transformation processes of recipient cells with the expression vectors using *Agrobacterium tumefaciens*, a gene gun or protoplast transfection, also including tissue culture impacts (e.g. see Allison K. Wilson, Jonathan R. Latham & Ricarda A. Steinbrecher (2006) Transformation-induced Mutations in Transgenic Plants: Analysis and BiosafetyImplications, Biotechnology and Genetic Engineering Reviews, 23:1, 209-238),
2.) occur during the genome editing process itself causing diverse unintended outcomes like off-target events, on-target effects at or in the vicinity of the target site, or large rearrangements of genomic regions, and
3.) are related to the effects of the intended changes at the target site(s) (including intervention in the metabolism and signalling pathways, intervention in ecosystem by disruption of inter-/intraspecies signalling, etc).
We reviewed the above mentioned risks and how they can be incorporated in the EU GMO risk assessment. These specific risks associated with genome editing techniques require the current examination of DNA to be expanded to encompass examination of epigenetic changes and changes in the transcriptome, proteome and metabolome of the GMO. Such examinations will also require further development of WGS (whole genome sequencing) and omics approaches, and may be assisted by new analytical tools in the future. These tools should be further developed to identify gene-edited crops, as also suggested by other researchers (Fraser, P.D. et al. (2020), Metabolomics should be deployed in the identification and characterization of gene-edited crops. Plant J., https://doi.org/10.1111/tpj.14679). Our results have been submitted in form of a review to Environmental Sciences Europe.

A few arbitrary examples of questions to be answered concerning unintended effects are:

- **What controls or triggers which repair mechanisms and how reliable are predictions?**
- **What happens if CRISPR/Cas expression vectors are present in an organism over a long time, including many generations? This is important for plants as well as for animals, and also has relevance to the understanding of impacts of engineered gene drives in a target species, in particular when resistance at the initial target site has occurred.**

b) Safety

Due to recent research showing that NGT-altered plants, animals and microbes, can exhibit unintended consequences at the expected site of alteration and elsewhere in the genome, it is imperative that all NGT-altered plants, animals and microbes undergo thorough safety assessments before they enter the food supply. This should include thorough genomic investigations, followed by long-term animal feeding studies using animals that are relevent to human health (e.g. rats) rather than farm animals that are not (e.g. chickens, trout and cows) and the studies should measure end-points that are relevant to human health.

c) Abuse and misuse of NGTs

As explained in our answers to questions 9 and 14 sub b, NGT research needs and gaps also result from cyber-overlaps, new risks, gaps in experience, exploitable safety/security measures, social engineering attacks, pretexting, counterfeiting, etc. Research into potential abuse and unintentional misuse of NGTs and NGT products resulting from this is being done by

ENSSER members. GMO counterfeiting methods, for instance, had been conceived before the advent of Crispr/Cas technologies, but Crispr/Cas turns them into high-security hazards.

d) Agriculture

Given the current rapid loss of biodiversity and the major contribution of current agricultural practices to biodiversity loss, ecosystem disruption and climate change, any publicly funded research should be in aid of remedying this as well as being in aid of creating resilient, sustainable, biodiverse and ecosystem supportive agricultural systems and enabling their practices.

The capacity of a (crop) plant to be a mutually supportive component in an agricultural ecosystem, i.e. capable of participating in communication and metabolic networks that provide resilience is equally crucial for modern and future agriculture. The push-pull system is a prime example of such resilient systems, as it fends off stemborer, fall armyworm and striga weed while at the same time protecting the soil against erosion and drying out, as well as providing nitrogen. The system (e.g. maize, napier grass and desmodium cover plants) was devised by researchers at ICIPE in Kenia and Rothamsted in the UK in a collaborative effort to serve local farmers. It requires intricate knowledge of system communication, i.e. of the action of semiochemicals, as well as of support networks. The capacity of plants to be part of such supportive networks (such as close communication and exchange with mycorrhizae) will need to become a focus of breeding as well as of farming practices. The impact of the use of NGTs will need to be tested on a case by case basis, which requires a very specific knowledge that is currently insufficient, pointing at another research gap.

Attachment 2 to questionnaire on NGTs
ENSSER

20. Could NGTs/NGT-products raise challenges/concerns for your sector/field of interest? *Yes/no*

- If yes, please describe and provide concrete examples/data.
- If yes, are these challenges/concerns specific to NGTs/NGT-products?
  - If yes, please explain.
  - If no, please explain why not.
- If no, please explain why not.

Yes. NGTs, esp. SDNs, are able to generate genomic alterations in plants which were not achievable with conventional plant breeding, nor with previous genetic engineering technologies.

The restriction of CRISPR/Cas9 due to its PAM requirement at the target sequence is constantly being reduced as many further alternatives to the classical Cas9-system are discovered and developed. Many plants have highly 'redundant' genomes, which means that they have many genes in multiple copies (as well as related pseudogenes) and most genes can be grouped in gene families based on sequence similarities. Gene family size is variable across different species and may have important functional outcomes related to speciation. Furthermore, many plant species are polyploid, meaning that they have more than two paired (homologous) sets of chromosomes. The guide RNAs are capable of recognizing all complementary target sequences present in the genome, irrespectively of how many gene copies are present. That means it is possible to alter at the same time all or multiple gene copies carrying the same target sequence in an organism. These alterations are not obtainable by using standard breeding techniques nor through naturally occurring mutations. Cas9 also enables the editing of different genomic sites using different guide RNAs in one organism simultaneously or successively in multiplexing approaches. CRISPR/Cas also allows the site-directed editing of closely situated base pairs, which is not possible through conventional breeding.

A high proportion of genes fall into linkage blocks that are resistant to recombination as most genes are located within the pericentromeric region and recombination predominantly occurs in distal regions of the chromosomes. Separation of linked genes is challenging and labour intensive using classical breeding methods. Site-directed nucleases can replace alleles in 'recombinogenic cold spots' fast, easily, and precisely leading to a combination of genetic material that would hardly occur naturally, thereby overcoming these natural limitations. In summary, the outcomes of these editing processes can result in organisms with new genetic combinations that would not occur naturally. Indicating all genomic alterations or allelic combinations generated by CRISPR/Cas9 generally as identical to naturally occurring variations is a misleading oversimplification. The possibilities of genome editing to generate crops with traits which have not yet been established by other methods are summarized in: Kawall K. 2019. New possibilities on the horizon: genome editing makes the whole genome accessible for changes. Front Pl Sci 10(525). doi:10.3389/fpls.2019.00525.

In conclusion: this results in a serious challenge to risk assessment, as summarised below.

NGTs pose challenges to current GMO risk assessment approaches as well as concerns over socio-economic and biosecurity matters. These are specific challenges and concerns for NGTs. Challenges and concerns are based on knowledge gaps and uncertainty:

- **A) novel uses (e.g. intentional changes in ecosystems/wild biodiversity)**
- **B) novel traits (e.g. underlying physiological mechanisms not yet sufficiently elucidated like those associated with de novo domestication of species)**
- **C) novel scale (performing NGTs can be scalable to modify entire population of species in vivo in the environment, e.g. environmental genetic engineering)**
- **D) unintended changes (arising from target modification, e.g. due to higher targeting efficiency; arising from off-target modification and analytical gaps in detection (e.g. modification of similar sequences in the entire genome or in the genome of several species at the same time))**
- **E) unintended changes from multiplexing techniques (combination of different techniques and multiplex applications, e.g. insertion and deletion of transgene sequences, transgenic dsRNA cassette, etc.)**
- **F) combinatorial and cumulative effects that arise through the release of different gene-edited organisms**
- **G) NGTs other than SDNs also pose challenges for risk assessment, as essential data, knowledge and understanding are still lacking (Eckerstorfer MF, Dolezel M, Heissenberger A, Miklau M, Reichenbecher W, Steinbrecher RA and 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. doi: 10.3389/fbioe.2019.00031)**
- **H) cyberbiosecurity uncertainties as explained in question 14 sub b.**

A challenge is equally the inability or difficulty to detect unintended effects or off-target effects:
For example, in a recent paper on CRISPR-cas9-mediated homology-directed repair (Skryabin et al. 2020; *Science Advances* doi: 10.1126/sciadv.aax2941) authors reported not just about the unintended insertion phenomenons (for both nonhomologous end joining and homology-directed repair) but also about the difficulty of detecting these. The authors state in their abstract: "Nevertheless, the rapidly evolving technique still contains pitfalls. During the generation of six different conditional knockout mouse models, we discovered that frequently (sometimes solely) homology-directed repair and/or nonhomologous end joining mechanisms caused multiple unwanted head-to-tail insertions of donor DNA templates. Disturbingly, conventionally applied PCR analysis, in most cases, failed to identify these multiple integration events, which led to a high rate of falsely claimed precisely edited alleles. We caution that comprehensive analysis of modified alleles is essential and offer practical solutions to correctly identify precisely edited chromosomes."

Attachment 3 to questionnaire on NGTs
ENSSER

24. What is your view on the safety of NGTs/NGT-products? Please substantiate your reply.

a) Technical concerns of risk assessment

It is not yet possible to confine the genetic change to the intended gene only. Thus, after the procedure, intended products must be separated from unintended products:

1. Only the changes intended

2. Changes only in the intended places in the organism

3. No changes in other organisms (environmental applications)

There are no protocols or consensus methodologies to perform this kind of analysis. In addition, the parameters determining the biochemistry and kinetics of CRISPR systems are mostly based on mammalian cell analysis and this might not reflect the activity of CRISPR systems in plants.

Natural mechanisms can overcome CRISPR gene disruptions. One third of verified gene knock outs with CRISPR still show residual protein expression owing to translational reinitiation or exon skipping. In some cases, frameshift mutation leads to retained gene activity. Frameshift mutations can lead to full disruption of the protein despite no or weak NMD – truncated RNA sequences present. The production of truncated proteins by alternative splicing and/or translation reinitiation is an important potential limitation of the CRISPR technology. Analytical tools to detect isoforms and to characterize knock-out lines generated using CRISPR–Cas9-induced frameshifts should be developed by high-resolution mass spectrometry (Nature Methods volume 16, pages1087–1093, 2019).

Not all sites are edited in a predictable manner. The precision of DNA editing is mainly determined by the fourth nucleotide upstream of the PAM site (predicted for HepG2 cells human cell type). Chromatin states affect editing of imprecise target sites (Chakrabarti et al., 2019, Molecular Cell 73, 699–713).

Kinetics and fidelity studies showed that the repair of Cas9-induced DSBs is not representative for the repair of naturally occurring DSBs indicating that natural processes are bypassed (Brinkman et al., *Mol. Cell* 70, 2018). In this light, our estimated error rates for Cas9-induced DSBs in the range of 20%–100% per break event seem rather high compared to the 0.33% natural rate from the study.

That two processes can produce similar products does not mean that all products of the two processes are the same and only the same (Heinemann JA. Expert scientific opinion on the status of certain new techniques of genetic modification under Directive 2001/18/EC. 2015. 46 pages. Available at: https://www.canterbury.ac.nz/media/documents/science-research/inbi/new-techniques-of-genetic-modification.pdf). Genome editing does not result in only a single reproducible mutation; it can be many mutations, in many organisms, at the same time.

Unintended changes arising from new technologies:

- ✓ **Where to look in the genome, transcriptome, miRNome..?**
- ✓ **Can intended and unintended DNA/RNA changes result in metabolic network disturbance?**
- ✓ **How robust is your analytical capacity to detect and identify unintended changes?**
- ✓ **Does it mean that all risk hypothesis must be related to the presence of foreign nucleic acids?**

b) Bias by conflict of interest

Within medical research, and particularly within pharmaceutical research, there have been a great many studies published over decades describing how safety studies on a particular product conducted by a company that wishes to make money out of that product, tend to find their product to be safe, whereas independent studies tend to find problems. This is why trying to publish a paper in a high-end medical journal such as The Lancet requires all authors to fill in an extensive conflict of interest statement that includes disclosing even minor benefits from companies, such as receiving funding to attend a conference. Consequently, there is ample evidence from other areas of medical research that NGTs and their products cannot be considered to be safe until safety assessments are conducted by people who are independent of those who would profit from NGTs. Furthermore, it is clear that such independent studies need to be done for each and every NGT or NGT product, and that a study on one NGT or NGT product does not confer safety to another one. Such studies have simply not been done. Until they are, there is no suitable evidence that NGTs or their products are safe.

c) Cyberbiosafety concerns

We have extremely little understanding of the safety of NGTs and NGT products, especially as these technologies are not happening in a vacuum. We do not know the vulnerabilities associated to the physical/chemical and cyber-overlaps, to automation and to the gap created between the digital and the actual product. A simple digital or cyber-based error could result in some slight shift or change of some laboratory parameters or conditions. Not all of these effects would be immediately visible – especially since it is possible that the cyber-based mechanisms themselves introduce vulnerabilities to quality testing routines etc. Why assume digital products always work as intended, especially when tied to other disciplines such as biotechnologies?

d) Safety issues of the application of NGTs for the environment:

› changes in the composition of plants, e.g. protein, starch or constituents with specific biological activity may impact the food web

› changes in the composition of plants may have an effect on biological characteristics with relevance for plant communication, including symbionts such as microorganisms and pollinators

› changes in the biological characteristics of the NGT organisms may affect their reaction to environmental stressors (biotic and abiotic) that may also alter their impact on ecosystems, especially if they potentially enhance fitness

› due to the potential of some organisms to persist and propagate in the environment, they can become invasive or cause disturbance and disruptive long-term effects.

e) Gene drives

Gene drives based on NGTs such as CRISPR-Cas carry all the safety concerns of NGTs plus an extra level of safety concerns due to (i) the impossibility of assessing their safety in a representative context without factually deploying them and (ii) our complete lack of knowledge about the consequences of the introduction of manmade gene drives into nature, which has never happened before.

Attachment 4 to questionnaire on NGTs
ENSSER

Question 26. What is your view on ethical aspects related to NGTs/NGT-products? Please substantiate your reply.

A major ethical issue is the fact that EU GMO legislation assesses the risks but not the benefits of GMOs, including NGT organisms. The claimed benefits of GMOs are the reason of their existence. If these claimed benefits would be assessed, a true consideration would take place of the question whether GMOs are wanted in agriculture, food and other sectors. They would be weighed against alternatives. The fact that GMOs, including NGT organisms, are authorised without any formal discussion of this, constitutes a bias of governance. In effect, this favours the production and marketing of GMOs, even though their risks are assessed. Thus, opponents of GMOs are at a disadvantage, which is an ethical problem.

Due to the application of NGTs in complex environmental systems in which the occurrence of serious damage is typically uncertain, the precautionary principle must be applied. The Swiss Federal Committee on Non-Human Biotechnology (ECNH, see ECNH 2018, 2019b) sets out in detail why it is highly relevant to go beyond the concept of precaution as laid down in environmental law and to examine the *ethical significance* and the *ethical justification* of precautionary measures in the environmental field. Precaution is a *morally significant* action-guiding principle in the regulation of new biotechnologies.

Other ethical aspects include considerations on risk ethics, responsibility in research and questions of nutrition and self-determination (ECNH 2016).

In this context, food is a key aspect of our lives, one which determines our understanding of ourselves and forms a major aspect of our identity, and is therefore seen as morally relevant. One essential expression of a person's self-determination is *freedom of choice*, which is considered as *a right to defend*. As a result of this right, we insist that also NGT-products should be labelled so as to provide information about their contents and production methods, giving consumers the freedom to choose to avoid these products.

The discussion about self-determination and food also includes other stakeholders, especially food producers. Seed producers, breeders and farmers must have the right to freely choose seeds, breeding methods and farming techniques. However, the production of seeds and the production and cultivation of crops are a prerequisite for ensuring individual self-determination with regard to food. To secure this right in the long term, the very foundation of food production must be protected: biodiversity in general and agrobiodiversity in particular, as well as arable land and sufficient water to cultivate crops.

The public authorities must ensure that the biodiversity and agricultural biodiversity necessary for food production are protected in the long term. As new biotechnologies like NGTs and their products, particularly if not regulated under current EU GMO regulations, threaten diversified GM-free seed production and hence agricultural biodiversity, the state is morally obliged to take all necessary measures to preserve the foundations of diverse and sustainable food production (ECNH 2016, 25f).

There is a close connection between the need to preserve and foster agrobiodiversity and research into plant breeding. If, due to the further development of NGTs, the type of research pursued by the private sector seems to be leading to a narrowing of the scope of the research objectives and, consequently, in the longer term to a reduction in agrobiodiversity, public funding of plant breeding research must strike a balance and ensure greater diversification in the research sector. Furthermore, developments in intellectual property and its impacts on research and objectives in plant breeding should be carefully monitored. If the developments in the field of NGTs have impacts on agrobiodiversity and the respect for self-determination that cannot be justified, intellectual property rights in plant breeding should be restricted. Relating to responsible research and NGTs, proper risk research needs to be conducted. Access to plant material must be guaranteed so that results can be assessed by independent third parties. Access to unpublished studies and studies with negative research results needs to be ensured (ECNH 2016, 30)

The organic sector, according to its principle of health, works within the boundaries of living organic nature with respect for the integrity of life and the integrated whole. Techniques that interfere directly at DNA level, e.g. NGTs, violate this integrity and are consequently not allowed in the organic sector.

Moreover, there is a moral duty to care for and protect the natural life-support systems as basis for the life of future generations. In 2010, the German Federal Constitutional Court declared in its ruling on the German Genetic Engineering Act: "In view of the fact that the state of scientific knowledge regarding the long-term consequences of the use of genetic engineering has not yet been finally clarified, the legislator has a special duty of care in which it must observe the mandate contained in Article 20a of the Constitutional Law to protect the natural foundations of life for future generations, too."

Reference:

ECNH/Federal Ethics Committee on Non-Human Biotechnology 2016: New Plant Breeding Techniques. Ethical Considerations. https://www.ekah.admin.ch/inhalte/ekah-dateien/dokumentation/publikationen/EKAH_New_Plant_Breeding_Techniques_2016.pdf

For more references, see answer to question 27.

Attachment 5 to questionnaire on NGTs
ENSSER

29. Do you have other comments you would like to make? *Yes/no*
- If yes, please provide your comments here.

One of our members is a former member of the German Parliament and Chairman of its Environment Committee and as such strongly advocates full transparency and rigorous technology assessment as a precondition of any significant application of NGTs.

There is a common misunderstanding expounded repeatedly by classical GM and NGT researchers and practitioners: it is, that these techniques are no different in principle from conventional breeding which we have been conducting for millennia. Of course it is the case that breeding alters genes, but the reverse argument, that therefore by altering genes one is doing the same as breeding, is technically incorrect and therefore wrong and misleading. The reason is that the selection involved in breeding also selects unwittingly for the developmental controls of the plant/animal as well as its epigenetic characteristics as indicated in qu. 15, 1/iii above. These latter are necessarily ignored in NGTs. NGTs are therefore fundamentally different from breeding, creating thereby ethical issues as well as regulatory questions.

NGTs are at the peak of current development of biotechnology; as with many other technical innovations, there are many considerations about how the technology serves the well-being of society. Much of technological development has been to expand the carrying capacity (CC) of the earth for humans; many aspects of CC have now been exceeded. For NGTs the question becomes: can they serve to further expand CC, or will they result in further damage to the biosphere and therefore become counter-productive? For the reasons given in the various questions above, it is likely that damage will exceed benefit; see for example Steffen, W. et al. (2018) Trajectories of the Earth System in the Anthropocene. www.pnas.org/cgi/doi/10.1073/pnas.1810141115 Our conclusion is that there are numerous existing alternative technologies that must be applied and that NGTs probably become more dangerous than merits their use; therefore NGT products must be labelled such that the public can choose whether or not to follow that route to sustainability.

One of our members has the experience from formerly working as a mathematician/cryptographer for 20 years, that it was never hard to convince anyone in this field that technologies (e.g. the cyber space) were susceptible to attack – and that this very possibility itself invited intrusions and crime. On the other hand, in the life sciences, where this member has worked in recent years, people are mostly ignorant about this, or they downplay it as conspiracy theories. This naivety is a serious risk in itself, especially with the many do-it-yourself kits available and NGTs becoming so easily accessible and widely applicable. NGTs have many unique features that make them susceptible to intended manipulations. Moreover, there is a fine line between intended manipulations and some of the unintended consequences fostered by cyber-overlaps.

Finally, we list some literature further supporting our answers:

Agapito-Tenfen SZ, Okoli AS, Bernstein MJ, Wikmark O-G, Myhr AI. 2018. Revisiting Risk Governance of GM Plants: The Need to Consider New and Emerging Gene-Editing Techniques. Front Plant Sci 9(1874). doi:10.3389/fpls.2018.01874.

Agapito-Tenfen, S.Z. and Wikmark, O.-G. Current status of emerging technologies for plant breeding: Biosafety and knowledge gaps of site directed nucleases and oligonucleotide-directed mutagenesis. GenØk Biosafety Report 2015/02.

Agapito-Tenfen, S.Z. Biosafety aspects of genome-editing techniques. TWN and ACB Policy Briefing November 2016.

Brinkman EK, Chen T, de Haas M, Holland HA, Akhtar W, et al. 2018. Kinetics and fidelity of the repair of Cas9-induced double-strand DNA breaks. Mol Cell 70(5): 801-813.e6. doi:10.1016/j.molcel.2018.04.016.

Eckerstorfer MF, Dolezel M, Heissenberger A, Miklau M, Reichenbecher W, Steinbrecher RA and 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. doi: 10.3389/fbioe.2019.00031

Eckerstorfer MF, Engelhard M, Heissenberger A, Simon S, Teichman H. 2019. Plants developed by new genetic modification techniques-comparison of existing regulatory frameworks in the EU and non-EU countries. Front Bioeng Biotechnol 7: 26.

Heinemann JA, Agapito-Tenfen SZ, Carman JA. 2013. A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. Environ Int 55: 43-55. doi:10.1016/j.envint.2013.02.010.

Heinemann JA, Walker S. 2019. Environmentally applied nucleic acids and proteins for purposes of engineering changes to genes and other genetic material. Biosafety and Health 1, 3, 113-123 (2019), https://doi.org/10.1016/j.bsheal.2019.09.003

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

Steinbrecher RA, Paul H. 2017. New genetic engineering techniques: precaution, risk, and the need to develop prior societal technology assessment. Environment 59: 38-47.

Wikmark, O.-G., Brautaset, T., Agapito-Tenfen, S.Z., Okoli, A.S., Myhr, A.I., Binimelis, R. and Ching, L.L. Synthetic biology: Biosafety and contribution to addressing societal challenges. GenØk Biosafety Report 2016/02, http://genok.com/wp-content/uploads/2016/12/Biosafety_report_16_02_web.pdf.