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European Commissioner for Health & Food Safety
Ms Stella Kyriakides

CC
Vice-President of the EU Commission
Mr Frans Timmermans

5 May 2023

The mandate of EFSA and unintended genetic changes caused by NGTs

Dear Ms Kyriakides,

Thank you very much for the reply that we received from Klaus Berend. We are writing to respond to his reply and to draw your attention to the new publication attached to this letter.

We all know that the issue of unintended genetic changes is crucial in the regulation of NGTs. We should, therefore, be aware that if hazardous unintended genetic changes are overlooked, they may spread rapidly within breeding populations and further accumulate by cross breeding. The consequences of such unintended genetic changes could potentially harm the environment as well as the future of plant and animal breeding and therefore pose severe risks to the food security of future generations.

In his letter, Mr Berend makes the following statement: "*I would like to recall that the Commission mandated EFSA to assess potential new hazards and risks of NGTs, and that in the context of the GMO framework this included risks associated to unintended effects at molecular and phenotypical level that NGTs could pose in comparison with conventional breeding or established genomic techniques.*
To reply to these mandates, EFSA has published, since 2012, several scientific opinions on plants produced by NGTs. All these opinions have indeed also addressed possible risks associated to unintended effects, taking into account the most recent scientific evidence."

As you may be aware, this information does not address our concerns. Please allow us, therefore, to explain our concerns in more detail: we pointed out in our letter that the site of the unintended genetic changes and the resulting genetic combinations caused by NGTs can be vastly different compared to those resulting from conventional breeding. Furthermore, unintended genetic changes can also include the insertion of transgenes which would not be expected to occur in conventional breeding. It is, therefore, not sufficient to just consider

the number (frequency) and the type of mutations (such as indels) when investigating the risks associated with genetic changes. To gain broader and more coherent knowledge regarding these issues, we suggested that EFSA should mandate key questions such as: '*Which unintended genetic changes caused by NGT processes are unlikely to occur with previously used methods (crossing and selection, random mutagenesis)?'* This question is not the only one which should be put to EFSA, but it exemplifies aspects that have so far been overlooked. According to the letter we received from Mr Berend, DG SANTE appears to believe that EFSA has dealt sufficiently with these questions in its opinions issued between 2012 to 2022. However, our overview below shows that this is not the case:

EFSA findings from 2012

In its opinions published in 2012, the EFSA dealt with the issue of unintended genetic changes. However, these findings only address the type of mutations (such as indels) and the frequency of mutations. As EFSA (2012a) concluded at that time: "*Whilst the SDN-3 technique can induce off-target changes in the genome of the recipient plant these would be fewer than those occurring with most mutagenesis techniques used in conventional breeding. Furthermore, where such changes occur they would be of the same types as those produced by conventional breeding techniques.*"

It should also be noted that the 2012 EFSA opinions are partially outdated, as the CRISPR/Cas technology was not available at that time. It also failed to consider important findings regarding genome organisation in the cells and the way in which CRISPR/Cas can escape repair mechanisms in the cells. Please see our attached publication for more information about what is really new in regard to NGTs.

EFSA findings from 2020

We appreciate that EFSA was asked to update its previous opinions from 2012. However, again, this EFSA opinion (2020) only deals with the frequency and type of mutations and does not consider criteria such as the site of the mutation or the resulting genetic combinations. As EFSA (2020) states in its summary: "*The EFSA Opinion on SDN-3 concluded that the application of SDN-3 can induce off-target mutations but these would be fewer than those occurring with most mutagenesis techniques. Where they do occur, these changes would be the same types as those derived by conventional breeding techniques (EFSA GMO Panel, 2012a). As SDN-1 and SDN-2 techniques use the same molecular mechanisms to generate DSB as SDN-3, the conclusions for SDN-3 are also applicable to SDN-1 and SDN-2.*"

We do acknowledge that EFSA (2020) points out that unintentionally inserted genetic information is relevant to risk assessment: "*When plant transformation is used to introduce the SDN module, the unintended insertion of plasmid DNA or other exogenous DNA into the plant genome can happen. Furthermore, the application of some methods (…) to achieve SDN-1 and SDN-2 modifications can result in the unintended integration of exogenous DNA whose sequence may be known a priori (…). If the final product is not intended to retain any exogenous DNA, the applicant should assess the potential presence of a DNA sequence derived from the methods used to generate the SDN modification (…) . It should be noted that the assessment of the unintentional integration of exogenous DNA is already part of the molecular characterisation in the risk assessment of GM plants, under EU Regulations. Therefore, this is not to be considered a new requirement for risk-assessing genome-edited plants.*"

It can be concluded from this EFSA statement that the risk assessment of unintended insertions of transgenes is not a new requirement in risk assessment - and it continues to be a relevant requirement. Consequently, both now and in future, it will be necessary for all plants derived from SDN processes to undergo molecular risk assessment, which should include the use of methods such as whole genome sequencing (WGS) to rule out the unintended insertion of transgenes. This finding is also supported by other experts. For example, we found it interesting that at an international conference on detection methods held in March 2023 in Berlin (https://www.bfr-akademie.de/gmo2023/), Mr. Dirk Schenke from the University of Kiel reported that unless whole genome sequencing is performed, we cannot be certain that a gene-edited plant is not transgenic. We agree with these findings, however, we are concerned that the EU Commission might not give it the necessary attention.

EFSA findings in 2021 and 2022 on synbio plants
In its opinions on plants derived from methods of Synthetic Biology, EFSA also discusses the examples of SDN wheat with reduced content in gluten (EFSA 2021) and a de-novo domesticated tomato (EFSA, 2022a). EFSA comes to the conclusion that both unintended metabolic changes and genetic effects which occur at the target sites may be associated with the intended trait. Consequently, there is an evident need for risk assessment in regard to these unintended effects. However, in regard to unintended genetic changes, EFSA neither considered the site of the mutation nor the resulting genetic combinations that may occur independently of the targeted trait.

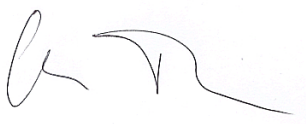
EFSA findings in 2022 on cisgenic plants
In its updated opinion on cisgenic plants, EFSA (2022b) again repeats that the frequency of mutations might be lower in the case of SDN-plants in comparison to previously used breeding methods. Again, EFSA did not take criteria such as the site of the mutation or the resulting gene combinations of unintended genetic changes caused by CRISPR/Cas processes into account. However, it does seem that EFSA has now become aware of the problem, as EFSA (2022 c) states that: "*Moreover, the GMO Panel was not mandated to provide a comprehensive literature review on the SDN-based technology and its unintended effects.*"

Conclusions from the EFSA findings
In regard to unintended genetic changes caused by SDN processes, EFSA has so far only considered the frequency of the mutation and the types of mutation that can be observed. However, EFSA neither explicitly nor implicitly considers the sites of the mutation or the resulting combinations of unintended genetic alterations. This is a major deficiency in the EFSA opinions: unless these criteria are applied, no conclusions can be drawn on the differences between unintended genetic changes caused by NGTs and those resulting from conventional breeding. Therefore, we feel the need to reiterate our request that EFSA addresses these specific questions to resolve such crucial issues.

Furthermore, in regard to the new publication (attached), we would like to draw your attention to further systemic risks that may be caused by NGT organisms being released into a shared environment. From this perspective, it is clearly the responsibility of the political decision-makers to keep control of all potential releases of NGT events, and thus avoid unintended interactions between those organisms with detrimental effects on health and the environment. Once again, when considering the potential interactions between NGT organisms, e. g. spontaneous crossings, it is self-evident that all unintended genetic changes have to be carefully risk assessed. We must ensure that we prevent hazardous unintended genetic changes, as these may well go unnoticed, and thus spread rapidly within breeding populations and further accumulate by cross breeding.

The only way to exclude or minimize these risks is in-depth molecular risk assessment of each NGT organism (including WGS and Omics) before any conclusion is drawn on its safety. Consequently, any hasty releases of NGT organisms or fragmentation of current EU GMO regulation must be avoided in order to prevent severe damage to future generations.

With kind regards



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Annex: Koller et al. (2023) The need for assessment of risks arising from interactions between NGT organisms from an EU perspective, https://enveurope.springeropen.com/articles/10.1186/s12302-023-00734-3