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Testbiotech comment on EFSA's draft updated opinion on plants developed through cisgenesis and intragenesis

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Introduction:

In June 2022, the EU Commission requested an "*updated scientific opinion on plants developed through cisgenesis and intragenesis*" from EFSA. The Annex to the request provides some background and terms of reference (TOR). The Annex (EU Commission 2021) itself appears to be partially biased towards political goals that are likely to impact the outcome of the opinion. In the background, for instance, the Commission states that:
"*Over the last ten years, following the requests by the European Commission, the European Food Safety Authority (EFSA) has issued scientific opinions on plants obtained through certain new genomic techniques (NGTs). (…) The main conclusions of the above mentioned opinions, relevant to the present mandate, are the following:*

- *Plants produced by SDN-1, SDN-2 and ODM techniques have no new hazards compared to conventionally bred and transgenic plants.*
- *Similar hazards can be associated with cisgenic and conventionally bred plants, while novel hazards can be associated with intragenic and transgenic plants. (…).*"

The EU Commission appears to assume that, based on previous EFSA opinions, it may be concluded that NGTs do not pose any new risks, regardless of whether they are compared to regulated technologies or non-regulated breeding methods. It further appears to assume, therefore, that all these technologies and breeding methods can be put in the same category. On the other hand, they also state that two different comparisons should be made in regard to cisgenesis and intragenesis: one comparison between risks associated with plants derived from cisgenesis and conventional breeding and the other between intragenic plants and transgenic plants. The question arises as to why a more specific comparison should be made for cisgenic and intragenic plants if all NGTs can simply be thrown together in the same category. The background provided by the EU Commission clearly needs clarification and correction. At the same time, none of the previous EFSA opinions lend support to this stratagem: it should be born in mind that no previous EFSA report, e.g. EFSA, 2012 or EFSA, 2020, has provided a full and comprehensive overview of the risks associated with NGTs. For example, the EFSA opinion on SDN-1 plants (EFSA 2020) explicitly states that no comprehensive literature research was conducted. In addition, although several publications describe the risks inherent to SDN technology (for overview see, for example, Kawall et al., 2020; Kawall 2021a; Kawall 2021b; Eckerstorfer et al., 2021; Testbiotech & CBAN, 2022), none of these are referred to in the EFSA opinions. The EU Commission meanwhile continues to create the impression that no new risks are associated with NGTs, regardless of whether they are compared to conventional breeding or transgenic plants, and thus appears to be giving a strong indication that the ultimate goal of their approach has from the outset been deregulation, with the deliberate intention of coming to flawed and highly misleading conclusions.

(1.4) EFSA interpretation of terms of reference

EFSA has fragmented and partially misinterpreted the questions posed by the Commission (EU Commission 2021). TOR1 very generally asks "*to identify potential risks that plants obtained by cisgenic and intragenic approaches could pose for humans, animals and the environment*." From our perspective, a much broader, unbiased survey would be needed to fulfill this requirement, and thus come to reliable conclusions. The EFSA approach of conflating TOR1 with TOR2 is suffering from its former opinions, as the methodology and accuracy of these previous findings and assumptions has not been sufficiently analyzed. This leads to confusion, because previous EFSA opinions were failing to deal with the risks inherent to the new processes of new genomic techniques (NGT) comprehensively. By referring to its previous opinions in the introduction, EFSA (2022a) reiterates (what is stated in the document of the Commission) that „ *Plants produced by SDN-1, SDN-2 and ODM techniques have no new hazards compared to conventionally bred and*

transgenic plants." From the outset it appears EFSA has concluded that NGTs do not pose any new risks, regardless of whether they are compared to regulated technologies or non-regulated breeding methods. However, directly afterwards it states that *"Similar hazards can be associated with cisgenic and conventionally bred plants, while novel hazards can be associated with intragenic and transgenic plants."* EFSA now appears to be assuming that two different comparisons should be made: one comparison between the risks of plants derived from cisgenesis and conventional breeding and the other between intragenic plants and transgenic plants. The question arises as to why a more specific comparison should be made for cisgenic and intragenic plants, if, on the other hand, all NGTs can just be collectively thrown into one category (which is apparently would be wrong). It is a question that is neither answered nor discussed anywhere in the text. This has the effect of introducing a major inconsistency throughout the opinion, similar to that observed in previous EFSA opinions (EFSA 2012, 2020, 2021). If the EFSA wants to avoid such confusion, it should answer the EU Commission question (TOR1) without fragmentation or conflation with TOR2. Comparisons with previous opinions may be useful, but should not be used as a starting point for the overarching question (TOR1). Consequently, answers relating to TOR1 and TOR2 should be given separately. TOR1 requires conducting a more comprehensive review, which could then be used to answer the other related TOR questions.

(2.4.1) Methodologies used by EFSA

According to EFSA, a literature review was conducted along the lines of specific criteria. Surprisingly, none of the selected publications included a report on cisgenic or intragenic products developed with new genomic techniques. In addition, only ten relevant patents were found. While Annex 1 presents some of the criteria used for the selection of the references, there appears to be no information available on which of the references were ultimately deemed relevant. Instead, most references included in the draft opinion are simply previous EFSA opinions; only very few publications were referenced, and they appear to have been chosen more or less arbitrarily. Another point is that the methodology appears to have changed (!) during the writing process so as to add some further references not included in the initial literature search results. Consequently, unless there is more transparency regarding the outcome of the research, the EFSA findings (2022a) cannot be assessed and no conclusions can be drawn. This also means that the results cannot be compared to the outcomes of other research, or to reports such as the one published by the JRC (2021), which refers to many examples of NGT plants. SDN-1 applications are included in the opinion if they cause "cisfragments" and "intrafragments". We would assume that such plants can, for example, be found in the JRC (2021) report. Beyond that, numerous reports and publications were published within last years which appear to create the impression that plants derived from NGTs will soon be brought to the market. It would be interesting to see how EFSA deals with specific examples, such as the GABA tomatoes introduced in Japan (Nonaka et al., 2017). Several NGT-plants appear to have already entered some non-EU markets, which may or may not be considered to be plants inheriting "cisfragments" or "intrafragments". Whatever the case may be, it is surprising that EFSA did not identify many more relevant products from the available sources. Furthermore, there are several publications dealing with the TOR1 question in regard to the risks of the technology and the resulting organisms (for overview see, for example, Kawall et al., 2020; Kawall 2021a; Kawall 2021b; Eckerstorfer et al., 2021; Testbiotech & CBAN, 2022). It is not clear whether EFSA took note of these findings. Consequently, we expect EFSA to substantially improve its methodology as well as to provide full transparency on its findings and on its selected/ rejected sources.

(3.1.1) Definition of ‘Established Genomic Techniques (EGTs)’

Using the expression ‘EGTs’ (as introduced by the Commission in its terms of reference) confuses the differences between regulated genetic engineering techniques (transgenic plants) and random mutagenesis as well as hybridisation techniques. If ‘EGT’ is used, it should be made clear which plants are genetically engineered (GE), and thus regulated, and which plants do not have to undergo the mandatory approval process and are, therefore, non-regulated. Furthermore, EFSA states “*with all the above-mentioned EGT techniques, the exogenous sequence integrates randomly at one or several positions in the genome, with potential consequences on the expression patterns.*” As a ‘stand-alone finding’ this sentence should be put into context. The random integration of additional genes may have many effects, and the EFSA should therefore also address other effects, e.g. epigenetic effects, the disruption of genes, genomic position effects, new and unintended open reading frames, the unintended introduction of additional genes and genomic interactions (including changes in gene expression) which may concern the plant constituents, changes in plant composition and agronomic characteristics of the plants (Windels et al., 2003; Makarevitch et al., 2003; Forsbach et al., 2003; Rang et al., 2005; Gelvin et al., 2017; Liu et al., 2019; Jupe et al., 2019; Yue et al., 2022).

(3.1.2) Assessment of ‘New Genomic Techniques (NGTs)’

The EFSA approach not only includes plants into which genes have been transferred and introduced into the cells, but also those generated with new genetic engineering techniques (New GE or NGT) using site-directed nucleases without the introduction of additional DNA (SDN-1). It seems that the plants have been divided into two groups: those with changes that add new genetic information to the gene pool of the species and those which do not (see 3.2.2.1.). However, it remains unclear as to how such conclusions can be drawn if the gene pool of a (potentially cross-able) species indeed comprises the genetic variants introduced by technical means into specific varieties. In any case, this draft opinion includes a much larger group of plants compared to the EFSA opinion (2012). It appears that the inclusion of SDN-1 and also SDN-2 plants extends beyond the EU Commission (EU Commission 2021) TORs dealing with cisgenesis (intragenesis) based on the transferal and introduction of additional gene sequences (which may, therefore, include SDN-3 plants only). According to this EFSA approach, all NGT processes may generate plants which are assumed to be cisgenic or intragenic. If this is the accepted approach, it would require the integration in the opinion of all relevant findings in regard to intended and unintended effects caused by SDN-processes (see, for example, Testbiotech & CBAN, 2022). It should be born in mind that EFSA has never provided a full and comprehensive overview in any of its previous reports (such as EFSA 2012 and EFSA 2020). For example, the EFSA (2020) opinion on SDN-1 plants explicitly states that no comprehensive literature research was conducted on this issue. In addition, several publications highlight the risks inherent to SDN technology (for overview see, for example, Kawall et al., 2020; Kawall 2021a; Kawall 2021b; Eckerstorfer et al., 2021; Testbiotech & CBAN, 2022) that are not referenced in the draft opinion. As long as EFSA simply continues to reiterate its position that NGTs pose no new risks, regardless of whether they are compared to conventional breeding or transgenic plants, the whole opinion is just an empty shell which fails to answer the TOR1 question, and can only provide flawed and highly misleading conclusions.

(3.2.1.1) What are the risks that cisgenic/intragenic plants could pose to humans, animals and the environment that were identified in the 2012 cisgenesis opinion?

This section again shows that the TOR1 question needs a comprehensive answer, without relying mainly on previous opinions and EFSA assumptions or on EFSA conflating TOR1 with TOR2: it is evident that even when the previous EFSA opinion on cisgenic plants was published in 2012, the EFSA findings and conclusions were not sufficiently backed by the science. For example, it was known (and also confirmed more recently) that insertional mutagenesis caused by transposons and retrotransposons is based on specific mechanisms which can also impact the sites of insertion and, in addition, many of these elements are integrated and 'domesticated' as regulatory elements into the plants' genome (Capel et al., 1993; Biémont & Vieira, 2006; Lisch & Bennetzen, 2011; Palazzo & Gregory, 2014; Servant & Deininger, 2016; Vicient & Casacuberta, 2017; Quadrana et al., 2019). It also should be taken into account that naturally occurring *Agrobacterium tumefaciens* only affects eudicotyledons (such as oil seed rape), while the modified bacteria can also be applied in monocotyledones (such as soybeans and maize). Therefore, the side effects of applications using the modified bacteria cannot be equated to those of its natural variants. Whatever the case may be, the mechanisms and results of these naturally occurring phenomena cannot be equated to the technical processes for the technical insertion of genes, such as biolistic methods and usage of *Agrobacterium tumefaciens*. For example, Yue et al. (2022), identified larger and smaller insertions as well as deletions caused by the biolistic method of gene insertion into papaya. The larger insertion consisted of 77 rearranged and translocated fragments, the larger deletion included 44 genes. More than 600 genes were changed in their activity. The changes caused by the method of genetic engineering could be clearly distinguished from other genomic changes which had occurred during the (around) 4000 years of the domestication of papayas. In conclusion, the processes used for the technical insertion of DNA can cause effects which are different in their scale, in the sites and in the patterns of the genetic change as well as their biological characteristics compared to those of non-regulated breeding methods or natural processes. This is also true if no additional genetic information is added to the gene pool of a species. Such effects may concern epigenetic regulation, the disruption of genes, position effects, open reading frames, the unintended introduction of additional genes, changes in gene expression and genomic interactions which can involve plant constituents, plant composition and agronomic characteristics (Windels et al., 2003; Makarevitch et al., 2003; Forsbach et al., 2003; Rang et al., 2005; Gelvin et al., 2017; Liu et al., 2019; Jupe et al., 2019; Yue et al., 2022). Such unintended effects have also been identified and discussed in many EFSA opinions in relation to applications for the import of transgenic plants (https://www.testbiotech.org/en/database). Therefore, these effects have to be assessed on a case-by-case basis to demonstrate safety of the plants as required by law.

(3.2.1.2) Is new information available that could impact the risk assessment of the products included in the EFSA 2012 opinion?

Recent research shows that – contrary to what was assumed by EFSA (2012) - the emergence of mutations is not completely random but influenced by gene regulation and genome organisation. Relevant factors that impact the likelihood of mutations are, for example, the composition of base pairs (Weng et al., 2019), histone modification (Lujan et al., 2015; Frigola et al., 2017; Belfield et al., 2018; Huang & Li 2018) and the status of chromatin (Gibcus & Dekker 2013; Luo, 2014; Guo & Fang, 2014; Gonzalez-Perez et al., 2019). Greater distances between specific genomic regions cause genes that may be particularly important for the survival of the species to mutate less frequently than others (Fang et al., 2008; Halstaed et al., 2020; Monroe et al., 2022). Gene regulation and genome organisation also have a substantial impact on the likelihood of repair

mechanisms in response to DNA damage (Belfield et al., 2018; Frigola et al., 2017; He et al., 2017; Kawall, 2019; Monroe et al., 2020). As a result, it is evident that the occurrence of mutations is not simply dependent on random processes followed by selection. Rather, gene regulation and genome organisation act as 'flexible safety barriers' in the evolution of plants. These findings are relevant for both Old GE ('EGTs') and New GE ('NGTs') and the resulting plants or products.
Furthermore, Barbour et al. (2022) show that a higher plant allelic diversity has an impact on different species within an experimental food web, and may play a crucial role in the stability of ecosystems and food webs. These effects are not caused by the introduction of new genetic information into the gene pool of a species, but by changing the frequency of the allelic variants within a population. Therefore, these effects are highly relevant to the effects caused by cisgenesis.
All in all, both Old GE and New GE can be the cause of biological effects which extend beyond those known from non-regulated breeding methods, even if no additional genetic information is added to the gene pool of a species. These intended or unintended effects may be different in their scale, in the sites and in the patterns of genetic change and their resulting biological characteristics compared to those of non-regulated breeding methods. Therefore, these effects have to be assessed on a case-by-case basis to demonstrate safety as required by law.

(3.2.1.3) Have new techniques/approaches been developed since 2012 that could be used to obtain cisgenic/intragenic plants as defined in the 2012 opinion?

EFSA points out that genes or gene variants (alleles) from wild relatives might become introgressed into domesticated varieties, as the use of new techniques, such as CRISPR/Cas, facilitates the transfer of isolated DNA sequences. However, EFSA does not give the 'full picture' of existing publications or of the effects that may be caused by these approaches which can, for example, involve multiplexing, i.e. targeting several genes at once within a single application (Raitskin and Patron, 2016; Zetsche et al., 2017). Although it is true, that gene linkage may be avoided with NGTs, this does not mean greater safety or predictability of the biological characteristics of the resulting plants. On the contrary, this may be associated with thus far not experienced or unexpected biological effects that may deserve the specific attention of the risk assessor (Kawall 2021).
Whenever additional genetic information is added to the gene pool of a species, the processes used for technical insertion of DNA can cause intended or unintended effects that extend far beyond what is already known from non-regulated breeding methods. Such effects may comprise epigenetic regulation, disruption of genes, new position effects, open reading frames, the unintended introduction of additional genes, changes in gene expression and genomic interactions which can involve plant constituents, plant composition and agronomic characteristics (see also Kawall et al., 2020; Kawall 2021). In addition, it has to be expected that the frequency and variety of genetic information which is present in the populations will be changed, thus also affecting the biological functions of what may be considered to be (but difficult to define more generally) 'keystone genes' (Barbour *et al.*, 2022). These intended or unintended effects may be different in their scale, in the sites and in the patterns of genetic change as well as their quality compared to those of non-regulated breeding methods (Kawall 2021, Eckerstorfer et al., 2021). Therefore, the intended and unintended effects have to be assessed on a case-by-case basis to demonstrate safety as required by law.

(3.2.1.4) If there are new techniques/approaches, what are the potential risks that may arise compared to those already covered in the 2012 opinion?

EFSA is completely wrong in suggesting that these plants do not carry any more risks than those identified in the 2012 opinion. Very generally, it is important to understand that a lower frequency of genetic change (if, for example, compared to methods used in random mutagenesis) does not imply greater safety of the specific intended or unintended changes arising from NGT applications (Kawall et al., 2021). SDN-2 and SDN-3 applications carry specific risks on several levels: (i) in many cases, the application requires a multistep process, including the production of transgenic plants in a first step. Potential risks associated with these processes were not sufficiently addressed in the 2012 opinion; (ii) tools such as CRISPR/Cas can, to varying degrees, escape the natural mechanisms of genome organisation. Therefore, the intended and unintended changes, the site of the integration, the patterns of genetic change and the resulting effects (that may come with risks) can exceed the effects already known from non-regulated breeding methods and Old GE; (iii) SDN-2 and SDN-3 processes cause specific unintended effects (often in the targeted genomic region, but also off-target) such as indels, larger genomic changes and unintended insertion of transgenes that would otherwise have been unlikely occur; (iv) if genetic linkages are separated or alleles of specific genes are made uniform (lowering the variety and frequency of the genetic variety in the population) this may cause genomic effects or biological phenomena which impact plant health, ecosystems and food safety. The same is true, if genetic information, which is within the gene pool of the species, is introduced into a new genetic background.

All these effects can carry specific risks which may be new in scale and quality compared to non-regulated breeding methods or Old GE. It is not possible to categorize NGT plants along specific ‘risk profiles’ to establish categories of plants which can be considered safe without in-depth risk assessment (Eckerstorfer 2021). If larger sections of NGT organisms (in terms of the number of organisms, traits and species) were to be exempted from regulation, the larger scale implies that at least some of these organisms may not be as safe as expected, thus potentially also having long-term implications which also may emerge from interactions with each other. Without sufficient regulation, there will also be no monitoring, no traceability and no sufficiently effective way of withdrawing the organisms from the environment in cases of urgency.

(3.2.2.1) What new products could be obtained using new approaches, in particular using SDNs, that could be classified as cisgenic/intragenic plants according to the definition given in the framework of this mandate?

EFSA has introduced a new concept in this section by expanding the concept of ‘cisgenesis’ to SDN-1 applications. It proposes that a ‘knocked-out’ gene function typical for SDN-1 applications results in so-called “cisfragements” that may already be within the gene pool of a species. This concept would have far-reaching implications: for example, Zsögön et al. (2018) show that the complexity of several introduced CRISPR/Cas-induced genetic changes results in a new quality of hazards and risks, even if no new genetic information is added to the gene pool of a species. In this case of ‘de novo domestication’, CRISPR/Cas9 is used to alter the genomes of wild species in such a way that some of their genes are modified to resemble domesticated ones. Such de novo domesticated plants still have some properties from wild species which were lost during plant breeding. While no new genes are added to the gene pool of the species, the plant composition and other biological characteristics of the plants may show pervasive changes that go beyond what was observed from previous GE. Therefore, plants altered with SDN-1 to achieve traits known from

cultivated varieties, but which are now expressed in a new genetic background, cannot be equated to their conventional or natural counterparts, as the corresponding target gene(s) might have divergent functions or interactions in different genetic backgrounds (see Kawall, 2021, EFSA 2022). This example (Zsögön et al., 2018) also shows that the dissecting of gene linkages does not mean greater safety or predictability of the biological characteristics of the resulting plants. This example again shows that a lower frequency of genetic changes (if compared to methods of random mutagenesis) in no way implies greater safety of the specific intended or unintended changes arising from NGT applications (Kawall 2021).

(3.2.2.2) What risks could those products pose to humans, animals and the environment compared to the risks associated with plants obtained from conventional plant breeding techniques or with EGTs?

Similarly to SDN-2 and SDN-3 applications, SDN-1 also carries specific risks on several levels: (i) in many cases, the application requires a multistep process, including the production of transgenic plants in a first step. The risks associated with these processes were not properly addressed in the 2012 opinion; (ii) tools such as CRISPR/Cas can, to varying degrees, escape the natural mechanisms of genome organisation. Therefore, the intended and unintended changes, the site of the integration, the pattern of genetic changes and the resulting effects (that may carry risks) can all cause the effects to extend beyond those already known from non-regulated breeding methods and Old GE; (iii) there are specific unintended effects arising from the processes of SDN-1 (often in the targeted genomic region, but also off-target) such as indels, larger genomic changes and unintended insertion of transgenes that would otherwise have been unlikely to occur; (iv) if genetic linkages are separated or alleles of specific genes are made uniform (lowering the variety and frequency of the genetic variety in the population), this may cause genomic effects or biological phenomena which impact plant health, ecosystems and food safety. The same is true, if genetic information, which is within the gene pool of the species, is introduced into a new genetic background. All these effects can occur along with specific risks which may be new in scale and quality compared to non-regulated breeding methods and/or Old GE (Kawall 2021).

Whatever the case, a lower frequency of genetic change (if compared to methods of random mutatgenesis) does not imply greater safety of the specific intended or unintended changes caused by NGT applications. Without in-depth risk assessment, it is not possible to categorize NGT plants according to specific ‘risk profiles’ and thus establish categories of plants which can be considered safe (Eckerstorfer 2021).

If larger sections of NGT organisms (in terms of the number of organisms, traits and species) were to be exempted from regulation and release in large scale, this implies that at least some of these organisms may not be as safe as expected, with potentially long-term implications which also may emerge from its interactions with each other. Without sufficient regulation, there will also be no monitoring, no traceability and no sufficiently effective way of withdrawing the organisms from the environment if this is urgently required. It should also be taken into account that NGT plants may be introduced and cultivated in large numbers and on millions of hectares within just a few years. Therefore, the potential scale of exposure to many different (in terms of traits and / or species) NGT plants, which have not adapted via evolutionary processes, has to be taken into account when it comes to the assessment of their overall environmental impact (Heinemann et al., 2021).

Furthermore, Barbour et al. (2022) show that a higher plant allelic diversity has an impact on different species within an experimental food web and may play a crucial role in the stability of ecosystems and food webs. These effects are not caused by the introduction of new genetic

information into the gene pool of a species, but by changing the frequency of the allelic variants within a population. CRISPR/Cas applications in particular can be used to make gene variants within a population more uniform, i.e. the frequency of the abundance of different allelic variants can be reduced, the alleles can be changed or the respective gene (-family) can be blocked in its functions. Therefore, these effects are highly relevant for the effects caused by New GE applications on cisgenesis and intragenesis. Whatever the case, EFSA is completely wrong in claiming that NGTs with 'cisfragments' and 'intrafragments' would not pose new risks compared to what was identified in the 2012 opinion (EFSA, 2012).

(3.3.1.1) Are the conclusions in the EFSA 2012 opinion on the applicability of the existing guidelines still valid, taking into account the newly published guidelines and the information made available since the publication of this opinion?

Based on its concept, EFSA includes (most) SDN-1, SDN-2 and SDN-3 applications in its opinion, and therefore plants considered to be 'synbio', e.g. the newly domesticated tomato (Zsögön et al., 2018, EFSA 2021 and 2022b), also appear to fall within its scope and will as such need to be fully considered. However, the EFSA draft opinion does not address any of these examples or other relevant cases and specific risks arising from the technical processes. Therefore, the EFSA draft opinion cannot be regarded as sufficient to derive final conclusions for TOR3. Furthermore, it seems that flexibility, as discussed by EFSA, is already present in the current system: the data requirements depend to some extent on the type and trait in the application (such as herbicide resistance, insect toxicity, changes in nutritional composition). In regard to the 90-day feeding studies requirement, EFSA has so far been unable to put forward any other (better) methodology to assess the potential effects emerging from whole food and feed, such as combinatorial health effects. Therefore, the demand to abandon mandatory feeding studies is not underpinned by any sufficient alternatives needed to demonstrate safety of whole food and feed. Nevertheless, it can be agreed that risk assessment practice does indeed require higher standards and more reliable methodology. This is especially true when it comes to NGT plants with genetic and phenotypical changes which go beyond the GE plants assessed so far. As shown, Old GE as well as New GE can cause biological effects well beyond those which are known from non-regulated breeding methods, even if no additional genetic information is added to the gene pool of a species. These intended or unintended effects may be different in their scale, in the sites and in the patterns of genetic change compared to those of non-regulated breeding methods. Therefore, these effects have to be assessed on a case-by-case basis to demonstrate safety as required by law.

(3.3.2.1) Are the existing guidelines for risk assessment fully or partially applicable, and are they sufficient to assess these new products?

Based on its concept, EFSA includes (most) SDN-1, SDN-2 and SDN-3 applications in its opinion, and therefore plants considered to be 'synbio' (EFSA 2021 and 2022b) also appear to fall within its scope and will as such need to be fully considered. However, as previously shown, the EFSA draft opinion does not address these examples, relevant cases or any specific risks arising from the technical processes. Therefore, the draft opinion cannot be seen as sufficient to derive final conclusions in regard to TOR3. More specifically, some of the conclusions presented by EFSA in its draft are flawed and misleading. For example, EFSA suggests that if "*the targeted introduction/modification of a gene to obtain an allele already existing within the species*" is used, "*these plants would not present new hazards as compared with conventional plants, and therefore most, if not all, risk assessment requirements would not be relevant.*" As shown, the effects of NGT

applications can present specific risks, which may extend in scale and quantity far beyond those already known from non-regulated breeding methods and/or Old GE. Without in-depth risk assessment, it is not possible to categorize NGT plants into specific ‘risk profiles’ in order to establish categories of plants which can be considered safe (Eckerstorfer 2021). It is completely inaccurate for EFSA to claim that NGTs do not pose a new scale and dimension of risk compared to plants derived from conventional breeding. Therefore, as with other regulated GE organisms, these plants must be assessed on a case-by-case basis to demonstrate safety as required by law. As shown, the risk assessment of these plants cannot be refined to the intended effects of the final products, it has to take into account unintended effects caused by the technical processes and the overall biological characteristics of the organisms.

(3.4.1) Which aspect (if any) of the existing guidelines should be updated, adapted or complemented?

Based on its concept, EFSA includes (most) SDN-1, SDN-2 and SDN-3 applications in its opinion, and therefore plants considered to be ‘synbio’ (EFSA 2021 and 2022b) also appear to fall in its scope and will as such need to be fully considered. However, as already shown, the EFSA draft opinion does not address any examples, relevant cases or specific risks arising from the technical processes. Therefore, the EFSA draft opinion cannot be seen as sufficient to derive to final conclusions in regard to TOR4. More specifically, some of the conclusions presented by EFSA in its draft are flawed and misleading (see 3.3.2.1). In regard to future guidelines, there is a need for the introduction of comprehensive methodology to assess changes in plant composition and phenotypic characteristics, which also makes use of ‘omics’ (genomics, transcriptomics, proteomics, metabolomics) and whole genome sequencing. In addition, the plants should be exposed to a sufficiently broad range of biotic and abiotic stressors to investigate the extent to which these factors impact plant composition, phenotypic characteristics and gene expression. In regard to toxicology, both the intended and unintended effects have to be considered. Apart from new proteins (peptides) which may be produced unintentionally, the emergence of other additional biologically active molecules (such as ncRNAs) and interactions with plant constituents, must also be considered. Furthermore, the impact on the immune system from the intestinal microbiome should, for example, also be taken into account. If applicants apply for approval to cultivate the plants, the guidelines should require the applicant to demonstrate that the plants cannot persist and propagate in the environment. Without introducing such ‘cut off criteria’ (Bauer-Panskus et al. 2020; Then et. al., 2020), environmental risk assessment cannot be conclusive. In addition, food webs and interactions with non-target organisms as well as the soil bacteria must be assessed in detail, and safety demonstrated through experimental data (for more information, see Testbiotech, 2021). Finally, the risk manager should develop examination guidelines to assess potential benefits to ensure that the only NGT plants used in agriculture and food production are those which are really necessary.

(4.) Conclusions

As shown, the EFSA draft opinion does not address any of the relevant cases and specific risks caused by the technical processes. Therefore, the EFSA draft opinion cannot be seen as sufficient to derive final conclusions in regard to the TORs provided by the Commission. More specifically, some of the conclusions presented in the EFSA draft are flawed and misleading. Beyond that, there is an evident need for the introduction of more comprehensive methodologies to assess the risks of plants obtained from Old GE and New GE.

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