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Assessment of genetically modified maize MIR162 for renewal authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-025), by Syngenta

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Introduction

The GMO Panel assessed maize MIR162 for renewal of authorisation (EFSA, 2022a). Maize MIR162 expresses VIP3a20 (for protection against certain lepidopteran pests) and PMI (selectable marker). According to EFSA, there is *"no evidence in renewal application EFSA-GMO-RX-025 for new hazards, modified exposure or scientific uncertainties that would change the conclusions of the original risk assessment on maize MIR162."*

1. Systematic literature review

The applicant provided a systematic review. This identified 50 papers relevant to risk assessment. However, the choice of studies is questionable since the review failed to consider a number of papers with greater relevance (see chapters below). Furthermore, the literature review makes no mention of new evidence concerning the effects of vip3 genes in maize (also see chapters below). The new evidence relates to a patent held by Syngenta, thus raising questions as to why this information was left out by the applicant, who should have been fully aware of its relevance.

2. Molecular characterisation

New bioinformatic data provided by the applicant showed a difference in the sequence of the event in new plant material compared to the sequence of the event in the originally assessed application (EFSA, 2012). The difference is located in a cytosine homopolymer region in the second of the two ZmUbiInt promoters contained in the MIR162 insert. EFSA found no risks to human or animal safety related to the nucleotide difference.

It should be noted that new evidence has emerged showing that vip3 genes seem to cause decreased male fertility in maize. In a patent (EP 3632202 B1, https://data.epo.org/publication-server/pdf-document?pn=3632202&ki=B1&cc=EP&pd=20220720) recently issued by the European Patent Office (EPO), the patent holder (Syngenta) claims that maize containing vip3 genes (such as maize MIR162) tends to show decreased fertility. The patent states:
"However, Vip3 has been observed to cause decreased male fertility in certain inbred maize plants under normal growing conditions. This phenomenon is more prominent in inbred maize plants that are homozygous for a vip3A transgene. The degree to which male fertility is decreased is inbred specific - some inbreds exhibit little or no reduction in male fertility when homozygous for a vip3 gene, other inbreds are somewhat sensitive to Vip3 and exhibit a significant reduction in male

fertility when homozygous for a vip3 gene, and other inbreds are highly sensitive to Vip3 and exhibit extremely low or no male fertility when homozygous for a vip3 gene. The degree to which male fertility is decreased is also affected by environmental factors, such as water availability and temperature. In Vip3-induced reductions in male fertility, drought and high temperature conditions exacerbate the reduction in male fertility; however, cooler growth conditions have been shown to mitigate the negative effects of Vip3 expression on male fertility."(EP 3632202 B1)

The applicant should have reported this surprising effect to EFSA, the underlying causes should have been investigated and assessed. The new scientific evidence raises several questions, for example:

- As the applicant most probably knew about the decreased male fertility in maize plants containing vip3 genes, why was this fact not reported to EFSA?
- What molecular mechanism is behind the decreased male fertility?
- What other traits of the maize plants may be compromised and how is this related to plant composition?
- To what extent is the genetic background of maize MIR162 impacted by this phenomenon?
- Under which climatic conditions may the said effect occur in maize MIR162?

Whatever the case, studies should now be conducted to investigate climatic conditions that might have an effect on maize MIR162. As stated in the patent*:*
"It was observed that an increase in environmental stress exacerbated the Vip3A-induced ructions in male fertility." Therefore, as the observed fertility reduction seems to be more pronounced under drought and high temperatures, it is a plausible hypothesis that climate change has to be considered a relevant factor in the risk assessments of all events containing vip3 genes (such as MIR162). Therefore, gene expression studies need to be conducted under a broad variety of environmental conditions and the results analysed using 'Omics' techniques.

Data from field trials show a range of mean values between 41 µg/g and 124 µg/g for VIP3Aa20 in the grain (Table 1), while in other cases 166 µg/g and more were measured as maximum range in the grain (EFSA, 2012). This is evidence of highly variable gene expression, with the actual content of the additional protein being unpredictable. Widely differing VIP3Aa20 contents were also found in stacked maize events comprising MIR162 (see Testbiotech, 2019). Against the backdrop of new evidence regarding fertility in different genetic backgrounds, it should be investigated whether the differences in gene expression of VIP3Aa20 might be associated with this unintended effect.

Table 1: Gene expression and content of Vip3Aa20 present in maize MIR162 in grain (µg/g dry weight, mean values)

Application (EFSA opinion)	Details from field trials	Content of VIP3Aa20
MIR162 (EFSA 2012)	Bloomington, Illinois 2005, Hybrid A	46
	York, Nebraska, 2005, Hybrid B	41
	Bloomington, Illinois, 2006, Hybrid A	124
	Bloomington, Illinois, 2006, Hybrid B	84
	Brazil, Ituiutaba, 2007	62
	Brazil, Uberlandia , 2007	59

3. Comparative assessment of plant composition and agronomic and phenotypic characteristics

The comparative assessment for renewal of MIR162 authorisation shows that data gaps were already present in the original EFSA opinion from 2012 (EFSA, 2012). Testbiotech (2012) requested more data for the field trials which were part of the original application at that time. Despite not being a requirement in EFSA guidance for renewal of authorisation, it has to be stated that: (1) no defined extreme weather conditions were taken into account; (2) not all relevant genetic backgrounds were taken into account.

In light of new evidence regarding decreased male fertility revealed in the Syngenta patent EP 3632202 B1, EFSA should have requested more data, especially on agronomic and phenotypic parameters, and tests with different genetic backgrounds. In this context, the compositional differences between maize MIR162 and its comparator, which were noted in the original application (EFSA, 2012), might also be considered to be effects of metabolic imbalances caused by the transgene.

The material taken from the plants should have been assessed by using 'Omics' techniques to investigate changes in the gene activity of the transgene and the plant genome, and also to investigate changes in metabolic pathways and the emergence of unintended biologically active gene products (see Benevenuto et al., 2022). Such in-depth investigations should not just be dependent on findings indicating potential adverse effects, they should always be necessary to draw sufficiently robust conclusions to inform the next steps in risk assessment.

As it stands, the data provided by the applicant and accepted by EFSA, are insufficient to conclude on the impact that environmental factors and genetic backgrounds may have on gene expression, plant metabolism or plant composition. It is also insufficient to conclude on agronomic and phenotypic characteristics. The plants should have been subjected to a much broader range of defined environmental conditions and stressors in order to gather reliable data on compositional analysis and agronomic characteristics.

Based on the available data, no final conclusions can be drawn on the safety of the plants.

4. Toxicity

EU legal provisions, such as Regulation 1829/2003, state that "*any risks which they present for human and animal health and, as the case may be, for the environment*" have to be avoided.

In the case of genetically engineered maize MIR162 expressing VIP3Aa20 toxin, there are still many open questions on specificity, mode of action and others, which may also be relevant to human or animal health. This is further reflected in comments made by experts from EU Member States (EFSA, 2022b), who conclude that the MIR162 risk assessment still cannot be finalised because important information is missing, in particular, on the specificity and toxicity of the insecticidal protein VIP3Aa20.

For example, there is a lack of understanding about the mode of action and binding of the VIP3Aa toxin. Testbiotech raised concerns in 2012 that in contrast to other Bt toxins, the toxicity of VIP3Aa20 does not appear to depend upon specific receptors (Lee at al., 2003). Interestingly, this lack of understanding has persisted until the present day, as shown in recent studies by Shan et al. (2022) or Quan (2022), which were not assessed by the GMO Panel. There is also evidence showing VIP3 proteins are less specific than Cry toxins (see Shan et al., 2022). In sum, this should have led to more scrutiny in the toxicological assessment of MIR162.

In regard to food and feed safety, EFSA (2019) considers microbiomes to be highly relevant to the health status of their hosts. Therefore, it is desirable to understand the importance of their role in risk assessment. EFSA expects gut microbiome research (not only in the case of GE plants) to play a relevant role in regulatory science with potential implications for future risk assessments and predictive risk models. As EFSA states: "*considering that the gut microbiome is a biological component directly and indirectly involved in the metabolism of food/feed components and chemicals and in the protection of the host against adverse environmental exposure, it would be useful to establish criteria on how to evaluate the potential adverse impacts of perturbators on this defensive barrier, and consequently, on human/animal health.*"

However, no attempts have been made to integrate the microbiome into the risk assessment of food and feed derived from the GE maize. This is in direct contradiction to Regulation 1829/2003 which requests "*genetically modified food and feed should only be authorised for placing on the Community market after a scientific evaluation of the highest possible standard, to be undertaken under the responsibility of the European Food Safety Authority (Authority), of any risks which they present for human and animal health and, as the case may be, for the environment.*" (Recital 9).

Furthermore, possible health effects related to the new scientific evidence regarding vip3 genes in maize cannot be ruled out on the basis of EFSA risk assessment.

5. Environmental risk assessment

The appearance of teosinte in Spain and France (see Testbiotech, 2016; Trtikova et al., 2017) is a further factor that needs to be considered. Maize volunteers can be found in the EU on a regular basis, as has been reported by Palaudelmàs et al. (2009) in Spain and by Pascher (2016) in Austria. Furthermore, the biological characteristics of the GE maize need to be examined in detail for next generation effects, volunteer potential (persistence) and gene flow. EFSA should also bear in mind that, according to a patent granted to the applicant, maize producing VIP3 toxins (such as MIR162) may show unexpected agronomic characteristics (reduction in male fertility in certain genetic backgrounds). Under these circumstances, even rare outcrossing events that go unnoticed may have long-term impacts on the agro-ecosystems. Points to consider include:

- Without more data on the teosinte species growing in the EU, the likelihood of gene flow from the maize to teosinte cannot be assessed (Trtikova et al., 2017). The same is true for gene flow from teosinte to genetically engineered plants.
- Furthermore, the characteristics of potential hybrids and next generations also need to be investigated and cannot be predicted simply from the data of the original event. It is well known that there can be next generation effects and interference from the genetic background that cannot be predicted from the assessment of the original event (Bauer-Panskus et al.,

2020). This issue is relevant to gene flow from maize to teosinte as well from teosinte to maize.

EFSA should have requested data from the applicant to show that no adverse effects can occur through gene flow from the maize to teosinte and / or from teosinte to the maize volunteers. In the absence of such data, the risk assessment and the renewal of authorisation have to be regarded as not valid.

Without detailed consideration of the hazards associated with potential gene flow from maize to teosinte and from teosinte to maize, no conclusion can be drawn on the environmental risks of spillage from the maize.

Testbiotech is aware of a recent statement issued by EFSA (2022c) regarding the teosinte situation in France and Spain. Here, EFSA comes to the conclusion:
"The new evidence retrieved confirms that where maize and EU teosinte plants co-occur and flower synchronously, maize alleles (transgenic or not), can move into teosinte populations at rates that depend on different factors. Hence, the possible introgression of transgenes from maize MON810, Bt11, 1507 and GA21 into EU teosinte may only provide a selective advantage to GM teosinte hybrid progeny under high infestation of target pests and/or when glufosinate-ammonium- and/or glyphosate-based herbicides are applied. However, this fitness advantage will not allow GM teosinte hybrid progeny to overcome other biological and abiotic factors limiting their persistence and invasiveness. Therefore, EFSA considers that the growth habits of EU teosinte plants and teosinte hybrid progeny are such that the acquisition of insect resistance and/or herbicide tolerance is unlikely to change their relative persistence and invasive characteristics under EU conditions."

However, even in the updated risk assessment, EFSA has still not considered next generation effects such as possible fitness advantages (see Bauer-Panskus et al., 2020). The updated teosinte risk assessment is, therefore, too narrow to conclude on possible environmental effects and provides no answers to relevant risk related questions.

6. Others

If approval for import is renewed, the applicant has to ensure that post-market monitoring (PMM) is developed to collect reliable information on the detection of indications showing whether any (adverse) effects on health may be related to GE food or feed consumption. Thus, the monitoring report should at very least contain detailed information on: i) actual volumes of the GE products imported into the EU, ii) the ports and silos where shipments of the GE products were unloaded, iii) the processing plants where the GE products was transferred to, iv) the amount of the GE products used on farms for feed and v) transport routes of the GE products. Environmental monitoring should be run in regions where viable material of the GE products such as kernels are transported, stored, packaged, processed or used for food/feed. In case of losses and spread of viable material (such as kernels) all receiving environments need to be monitored. Furthermore, environmental exposure through organic waste material, by-products, sewage or faeces containing GE products during or after the production process, and during or after human or animal consumption should be part of the monitoring procedure.

We agree with comments made by experts from Member States (EFSA, 2022b) that the monitoring plan requires improvements such as those suggested by the German authority, BVL.

7. Conclusion

According to new evidence regarding vip3 genes in maize, maize MIR162 may show unintended effects like decreased male fertility. This evidence was neither reported by the applicant (who owns a patent claiming this effect) nor assessed by EFSA. The non-reporting should be investigated. Further, as field trial data showed major variations in transgene expression and many significant differences in plant composition that may be attributed to the unintended effect described in the applicant's patent, safety of MIR162 can not be concluded at this stage. New data should be requested and assessed by EFSA.

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