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Assessment of genetically modified Maize MON 87429 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2019-161) by Bayer

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Introduction

The GMO Panel assessed the herbicide tolerant maize MON 87429 (EFSA, 2022a). This event was developed to confer tolerance to dicamba, glufosinate, quizalofop and 2,4-D herbicides. Further, the maize expresses the CP4-EPSPS protein and utilises an endogenous maize RNAi regulatory element to suppress its expression in pollen. This results in male sterility when MON 87429 plants are exposed to glyphosate at a certain growth stage. This is part of a hybridisation system to be used in inbred lines to facilitate the production of hybrid seeds. According to the applicant, if glyphosate is applied outside specific growth stages, this does not lead to male sterile plants but will reduce plant yield.

1. Systematic literature review

A systematic review as referred to in Regulation (EU) No 503/2013 was not provided by the applicant. Based on preliminary information, the GMO Panel agreed that there was only limited value in undertaking a systematic review (EFSA, 2022a). One relevant scientific paper (written by Monsanto scientists) was identified by contractors hired by the applicant. However, a wider range of scientific literature is available on most of the herbicide tolerance traits present in maize MON 87429. The literature search is therefore not acceptable. Criticism was also voiced by Member States' experts (EFSA, 2022b). *"The petitioner describes the criteria used to select the articles, but these criteria are found to be too narrow by the 'Biotechnology' WG because they are limited to MON87429 maize, a recent occurrence. The petitioner has called upon 3 reviewers (1 internal and 2 external reviewers) to conduct this analysis independently. The 'Biotechnology' WG regrets that the body to which these reviewers belong is not disclosed, which makes it impossible to judge their level of independence from the applicant. There is no mention of a test of analytical consistency between the three reviewers."*

2. Molecular characterisation

Gene products, such as unintentionally produced ncRNA (non-coding RNA) from additional open reading frames, were not assessed. Thus, uncertainties remain about other biologically active substances resulting from the method of genetic engineering and the newly introduced gene constructs.

Environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). More specifically, Fang et al. (2018) showed that stress responses especially can lead to unexpected changes in plant metabolism, if they inherit additional EPSPS enzymes. However, the expression of the additional enzymes was only measured under field conditions in the US for one year. It is unclear, to which extent specific environmental conditions will influence the overall concentration of the enzymes in the plants. The plants should have been subjected to a much broader range of defined environmental conditions and stressors to gather reliable data on gene expression and functional genetic stability.

Due to increased weed pressure, it has to be expected that these plants can and will be exposed to high and also repeated dosages of complementary herbicides. Higher applications of herbicides will not only lead to a higher burden of residues in the harvest, but may also influence the expression of the transgenes or other genome activities in the plants. This aspect was completely ignored in risk assessment.

EFSA should have requested that the applicant submit data from field trials with the highest dosage of the complementary herbicides that can be tolerated by the plants, also including repeated spraying and the application of each of the relevant herbicides alone and in combination. The material derived from those plants should have been assessed by using 'Omics' techniques to investigate changes in the gene activity of the transgene, as well as the natural genome of the plants.

Data on herbicide application rates and their impact on gene expression
From the available information, it appears that the complementary herbicides were only applied in combination, and only sprayed once. Nevertheless, EFSA is of the opinion that the design of the field trials is in accordance with the expected agricultural practices. To justify this opinion, EFSA should have presented more detailed reasoning.

Current EFSA practices are such that it is not possible to access the original data submitted by the companies within the period of consultation. Therefore, the opinion has to provide all the data necessary to allow other experts to conclude whether the provisions of GMO regulation are fulfilled.

In light of the information available, we assume that the application and the data provided do not sufficiently represent the agricultural practices, which could include the use of single herbicide applications, higher dosages and repeated spraying.

Therefore, EFSA should have requested the applicant to submit data from field trials that included all the relevant agricultural practices, all active ingredients, all dosages and all combinations of the complementary herbicides that might be used in the agricultural practice of the GE maize producing countries. Without these data, no reliable conclusion can be drawn as requested in Implementing Regulation 503/2013 (in particular for herbicide tolerant GE plants) to assess whether anticipated agricultural practices influence the expression of the studied endpoints (see also Miyazaki et al., 2019).

Consequently, the GE maize plants tested in field trials do not sufficiently represent the products intended for import. The data presented by the applicant are insufficient to conclude on the impact of the herbicide applications on gene expression, plant composition or biological characteristics of the plant as requested in EU Regulation 503/2013.

Impact of genetic backgrounds on gene expression
It is known that the genomic background of the varieties can influence both the expression of the inserted genes and plant metabolism (see, for example, Lohn et al., 2020; Trtikova et al., 2015). However, it appears that the data on gene expression were confined to a single variety. Therefore, EFSA should also have requested additional data from transgenic maize varieties, e.g. those cultivated in South America.

However, EFSA has not taken these issues into consideration. Consequently, the GE maize plants tested in field trials do not sufficiently represent the products intended for import. The data presented by the applicant are therefore insufficient to conclude on the impact of the genetic backgrounds on gene expression as requested in EU Regulation 503/2013.

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

Implementing Regulation 503/2013 requests:
"*In the case of herbicide tolerant genetically modified plants and in order to assess whether the expected agricultural practices influence the expression of the studied endpoints, three test materials shall be compared: the genetically modified plant exposed to the intended herbicide; the conventional counterpart treated with conventional herbicide management regimes; and the genetically modified plant treated with the same conventional herbicide management regimes.*"

"*The different sites selected for the field trials shall reflect the different meteorological and agronomic conditions under which the crop is to be grown; the choice shall be explicitly justified. The choice of non-genetically modified reference varieties shall be appropriate for the chosen sites and shall be justified explicitly.*"

The data presented by Bayer do not meet the requirements of Implementing Regulation 503/2013: (1) the field trials were not conducted in all relevant regions where the GE maize will be cultivated, and not all relevant extreme weather conditions were taken into account (such as drought); (2) the field trials did not take all relevant agricultural management practices into account; (3) not all relevant genetic backgrounds were taken into account.

Data on environmental factors and stress conditions - and their impact on plant composition and phenotype
Field trials to assess plant composition as well as agronomic and phenotypic characteristics of the GE maize were only conducted in the US for one year. No extreme weather conditions were reported from the field trials, so no conclusions to be drawn on how gene expression will be affected by more severe climate stress due to drought, watering or high temperatures. In order to assess changes gene expression, the plants should have been grown in various environmental conditions and exposed to well-defined environmental stress conditions. This requirement is especially relevant in this case, since it is known that the additional epsps gene may show pleiotropic effects, also affecting seed dormancy, growth and stress responses of the plants (see, for example, Fang et al., 2018; Wang et al., 2014; Yang et al., 2017; Beres et al., 2018, Beres, 2019).

It should not be overlooked that, for example, Brazil is among the most important countries for maize imports into the EU: Brazil is a major producer of genetically engineered maize and is one of the largest exporters of maize to the EU (Commission Committee for the Common Organisation of Agricultural Markets, 2021).

Nevertheless, EFSA is of the opinion that the design of the field trials is in accordance with the expected agricultural practices. To justify this opinion, EFSA should have provided a much more detailed reasoning. Due to current EFSA practices, it is not possible to access the original data from the companies within the period of consultation. Therefore, the opinion has to provide all the necessary data to allow other experts to conclude whether the provisions of GMO regulation are fulfilled. In light of the information available, we assume that the application and the data provided do not sufficiently represent the agricultural practices and bio-regional conditions under which these plants are likely to be grown.

No experiments were requested to show to which extent specific environmental conditions influence plant composition and agronomic characteristics. Hence, no data were made available as requested in Implementing regulation 503/2013 to assess whether the expected environmental conditions under which the plants are likely to be cultivated will influence the expression of the studied endpoints.

Data on herbicide application rates and their impact on plant composition as well as agronomic and phenotypic characteristics

Due to the mode of action of the active ingredients in the complementary herbicides, it is plausible that complementary herbicide applications will cause stress responses in the plants, and thus impact gene expression and plant composition. These effects may vary with the amount of herbicide sprayed onto the crop and the various active ingredients which can be used.

From the available information, it looks like that the complementary herbicides were only applied in combination, with only one post-emergent (during the growth of the plants) spraying. Nevertheless, EFSA is of the opinion that the design of the field trials is in accordance with the expected agricultural practices. To justify this opinion, EFSA should have provided a much more detailed reasoning. Due to current EFSA practices, it is not possible to access the original data from the companies within the period of consultation. Therefore, the opinion has to provide all necessary data to allow other experts to conclude whether the provisions of GMO regulation are fulfilled. In light of the information available, we assume that the application and the data provided do not sufficiently represent the agricultural practices, i.e. single herbicide use, higher dosages and repeated spraying.

EFSA should have requested the applicant to submit data from field trials on all the relevant active ingredients used in agricultural practice, including all dosages and combinations of the complementary herbicides which might be used in agricultural practice in GE maize producing countries. Without these data, no reliable conclusions can be drawn as requested in Implementing Regulation 503/2013 (in particular for herbicide tolerant GE plants) to assess whether anticipated agricultural practices influence the expression of the studied endpoints (see also Miyazaki et al., 2019).

Consequently, the GE maize plants tested in field trials do not sufficiently represent the products intended for import. The data presented by the applicant are insufficient to conclude on the impact of

the herbicide applications on gene expression, plant composition or biological characteristics of the plant as requested in EU Regulation 503/2013.

Impact of genetic backgrounds on plant composition as well as on agronomic and phenotypic characteristics

Only 9 agronomic and phenotypic endpoints were subjected to statistical analysis. Whereas no significant differences were found between the isogenic line and MON 87429 not treated with the complementary herbicides, 3 endpoints were significantly different in plants sprayed with the complementary herbicides, indicating effects caused by the use of the herbicides.

It is known that the genomic background of the varieties can influence both the expression of the inserted genes and plant metabolism. However, it appears that the data on gene expression were confined to a single variety. Therefore, EFSA should also have requested additional data from transgenic maize varieties that are, for example, cultivated in South America.

However, EFSA has not taken these issues into consideration. Consequently, the GE maize plants tested in field trials do not sufficiently represent the products intended for import. The data presented by the applicant are therefore insufficient to conclude on the impact of the genetic backgrounds on gene expression as requested in EU Regulation 503/2013.

Data from compositional analysis show the need for further investigations

63 constituents were subjected to statistical analysis (9 in forage and 54 in grain):

- Statistically significant differences between the maize not treated with the complementary herbicides and the non-GM comparator were identified for 18 endpoints (equivalence category I or II)
- Statistically significant differences between the maize treated with the complementary herbicide and the non-GM comparator were identified for 18 endpoints (equivalence category I or II); levels of ADF, NDF and TDF in grain of treated GM maize (according to Table 3) fell under equivalence category III.

Given the above reasoning on the impact of environmental factors, herbicide applications and genetic backgrounds, as well as a higher number of significant findings in fields treated with the complementary herbicides, EFSA should indeed have requested more data: data on agronomic and phenotypic endpoints should be generated from a wider range of clearly defined stress factors, including all relevant agricultural practices and genetic backgrounds. This requirement is especially relevant in this case since it is known that the additional epsps genes may show pleiotropic effects, which also affect seed dormancy, growth and stress responses of the plants (see, for example, Fang et al., 2018; Wang et al., 2014; Yang et al., 2017; Beres et al., 2018, Beres, 2019).

Furthermore, as mentioned by experts from Member States (EFSA, 2022b), findings by Christ et al. (2017) showing that the PAT/BAR enzyme may also acetylate endogenous amino acids, should have been the starting point for further investigations.

A more detailed analysis would have been necessary to investigate changes in plant composition and phenotype, and also to investigate potential unintended changes in metabolic pathways and the emergence of unintended biologically active gene products.

The material derived 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 depend on findings indicating potential adverse effects, they should always be necessary to draw sufficiently robust conclusions to inform the next steps in risk assessment.

In addition, in awareness of the absence of any independent data on this maize (see literature review, EFSA, 2022a), we strongly recommend establishing a system with independent controls to repeat the trials and double check the data on plant composition and agronomic characteristics.

Conclusion on the comparative assessment of plant composition as well as on phenotypic and agronomic characteristics

The data provided by the applicant and accepted by EFSA are insufficient to conclude on the impact of environmental factors, herbicide applications and genetic backgrounds on gene expression, plant metabolism, plant composition, or on agronomic and phenotypic characteristics.

To gather reliable data on compositional analysis and agronomic characteristics, the plants should have been subjected to a much broader range of defined environmental conditions and stressors. Furthermore, EFSA should have requested the applicant to submit data from field trials which reflect current agricultural practices, including all relevant complementary herbicides and all relevant genetic backgrounds.

However, only samples from field sites located in the US were used to generate the data, and the impact of environmental factors and agricultural practices were not assessed in detail. Herbicide applications in the field trials did not represent all the relevant agricultural practices. Only one transgenic variety was grown in the field trials.

Consequently, the data presented by the applicant and accepted by EFSA are insufficient to conclude on the impact of environmental factors, herbicide applications or different genetic backgrounds on plant composition and agronomic characteristics.

Based on the available data, no final conclusions can be drawn on the safety of the plants. Therefore, the data neither fulfill the requirements of Implementing Regulation 503/2013 nor Regulation 1829/2003. This is also underlined by several statements made by experts from Member States (EFSA, 2022b).

In summary, the GE maize plants tested in the field trials do not sufficiently represent the products intended for import.

4. Toxicity

Implementing Regulation 503/2013 requests:

"*Toxicological assessment shall be performed in order to:*

(a) demonstrate that the intended effect(s) of the genetic modification has no adverse effects on human and animal health;

(b) demonstrate that unintended effect(s) of the genetic modification(s) identified or assumed to have occurred based on the preceding comparative molecular, compositional or phenotypic analyses, have no adverse effects on human and animal health;"

"*In accordance with the requirements of Articles 4 and 16 of Regulation (EC) No 1829/2003, the applicant shall ensure that the final risk characterisation clearly demonstrates that:*
(a) the genetically modified food and feed has no adverse effects on human and animal health;"

In addition, Implementing Regulation 503/2013 requests:

> *"For silencing approaches by RNAi expression, potential 'off target' genes should be searched by in silico analysis to assess if the genetic modification could affect the expression of other genes which raise safety concerns."*

Findings from molecular characterisation and comparative approach
As explained above, many significant changes in plant composition were identified. Even if the changes taken as isolated data might not directly raise safety concerns, the overall number of effects should have been considered as a starting point for much more detailed investigation into their potential health impacts.

However, the data presented by the applicant did not take into account cultivation of the maize under more extreme drought conditions, i.e. neither under realistic agricultural conditions nor considering all relevant countries of cultivation. The range of differences and their significance are likely to be substantially increased in these conditions. Thus, without more data, the true range of unintended effects cannot be determined and safety cannot be demonstrated as requested by EU regulation.

Findings from a 90-day feeding study
A 90-day subchronic toxicity study was conducted by the applicant.
Three groups of 16 male rats and 16 female rats were fed with diets containing:
- 50% (p/p) of ground maize grains of MON87429,
- 33% (p/p) of ground maize grains of MON87429 with 17% of isogenic non-GE maize,
- 50% (p/p) of ground isogenic non-GE maize,

MON87429 maize used in this study was treated with the four complementary herbicides (glufosinate-ammonium, dicamba, quizalofop, 2,4-D).

According to EFSA, "no treatment related adverse effects were observed in rats after feeding diets containing MON84279 grains up to/at 50% maize for 90 days."

However, experts from Member States voiced several concerns about the study (EFSA, 2022b):

- "the power calculation presented by the petitioner is not valid. This calculation is in fact carried out for only eight parameters, and the effect sizes selected by the petitioner without substantiation (e.g. 200% for cholesterol, 100% for alkaline phosphatase or 50% for creatinine) are not considered to be appropriate. For information purposes, the US EPA (2002) points out that effect sizes should generally be between 10 and 25%. Moreover, the 'Biotechnology' WG notes a greater variability for some parameters in this study compared with the historical data of the investigating centre, which further limits the statistical power of this study."
- "Currently available data on 90 Days Feeding Study in Rats with both 33% MON 87429 Test

Diet (low) and 50% MON 87429 Test Diet (high) MON 87429 Maize showed statistically significant influence on metabolic and hormonal parameters arise serious concerns to justify MON 87429 as a safe food and feed material in long-term use."

EFSA's response to the concerns remains unsatisfactory. For example, the Agency simply states that "the outcome of the statistical analysis were considered adequate by the EFSA GMO Panel". However, a critical assessment of the experts' questions is missing.

Effects of residues from spraying with complementary herbicide specific to GE plants and their mixed toxicity

The residues from spraying were considered to be outside the remit of the GMO Panel. However, without detailed assessment of these residues, no conclusion can be drawn on the safety of the imported products: due to specific agricultural management practices in the cultivation of the herbicide-resistant plants, there are, for example, specific patterns of spraying, exposure, occurrence of specific metabolites and emergence of combinatorial effects that require special attention.

EU pesticide regulation and GMO regulation both require a high level of protection for health and the environment. Thus, in regard to herbicide-resistant plants, specific assessment of residues from spraying with complementary herbicides must be considered a prerequisite for granting authorisation.

EU legal provisions, such as Regulation 1829/2003 (and Implementing Regulation 503/2013), 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. Therefore, potential adverse effects resulting from combinatorial exposure of various potential stressors need to be tested for mixed toxicity (EFSA, 2019b).

The agricultural use of glyphosate is not included in the application. Data on quizalofop and dicamba seem to be scarce. 2,4-D and glufosinate have been shown to impact or disturb the microbiome, which can have substantial impact on the long-term toxicity (mixed toxicity) of whole food and feed derived from the maize. In addition, glufosinate is classified as showing reproductive toxicity (http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN) and there are indications of additive or synergistic effects of the residues from spraying (Reuter, 2015). Dong et al. (2020) show that glufosinate can severely impact the microbiome; Tu et al. (2019) provide evidence on the adverse effects of 2,4-D.

In general, the microbiome can be seen as a common network of life, encompassing and closely interacting with plants, animals and humans. Microbial networks are thought to have co-evolved with their hosts and have developed a mutualistic relationship that benefits both the host and microorganisms. They act at the interphase and communicate between the organisms and their wider environment while at the same time being part of an organism's closer environment. Microbiomes are considered to be vital for the health of higher organisms, i.e. humans, animals and plants.

Therefore, potential adverse effects resulting from the exposure to whole food and feed need to be tested for mixed toxicity (EFSA, 2019b). This should also be considered in regard to changes in the intestinal microbiome. For example, Liao et al. (2021) describe effects of dicamba on soil organisms, causing prevalence of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in soil microbiomes. Similar or different effects may also be relevant for the intestinal

microbiome at the stage of consumption and should, therefore, be taken into account for dicamba resistant GE plants. The described effects, which may enhance the uptake of DNA from the transgenic plants by gut bacteria, are not considered under pesticide regulation, they have to be assessed within GMO risk assessment. The reason: these effects are highly dependent on the specific dosages applied on the GE plants, as well as on their metabolism and the resulting pattern of exposure in food and feed. In addition, cumulative effects (mixtures of GE plants in one diet) may play a decisive role. Under Directive 2001/18/EC, such effects could be considered to be indirect effects which may be immediate, delayed or cumulative. Implementing Regulation 503/2013 (point 1.4.2) requires "testing of new constituents other than proteins". In our opinion, this requirement also includes the assessment of residues from the complementary herbicides, which necessarily become constituents of all genetically engineered plants resistant to them.

In regard to food and feed safety, EFSA (2020) 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 that gut microbiome research (not only in the case of GE plants) will 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.*"

A 2019 study commissioned by EFSA on adjuvanticity / immunogenicity assessment of proteins included the role of the microbiome. Parenti et al. (2019) state that "*one of the most important drivers of immune response is the gut microbiota and other microbial constituent of the human body which are able to regulate host-pathogen balance and to produce systemic pro-inflammatory stimuli. The lifelong antigenic load represented by foods and bacteria/bacterial products leads to a profound remodeling of the gut microbiota and these changes are emerging as a driving force of the functional homeostasis of the immune system. As a matter of fact, a perturbation of the gut microbiota homeostasis due to irregular lifestyles, stress and age may lead to gut microbiota dysbiosis. This condition may predispose the host to metabolic disorders and inflammation*."

These findings are highly relevant to the risk assessment of the GE maize, which inherits combinations of herbicide resistance to dicamba, quizalofop, glufosinate and 2,4-D (and glyphosate, for certain uses). These residues may cause gut microbiome perturbation, depending on exposure and combinatorial effects. It has to be considered a plausible hypothesis that the effects on the microbiome can trigger effects on the immune system, food uptake and body weight. This hypothesis and mixed toxicity need to be tested before any conclusion can be drawn on the health safety of food and feed. Since no such data can be derived from pesticide risk assessment, experimental data on mixed toxicity of the maize have to be requested from the applicant.

In general, antibiotic effects and other adverse health effects might occur from exposure to a diet containing these plants that were not assessed under pesticide regulation. These adverse effects on health might be triggered by the residues from spraying with the complementary herbicide. Further attention should be paid to the specific toxicity of the metabolites of the pesticide active ingredients.

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).

EU legal provisions such as Regulation 1829/2003 (as well as Implementing Regulation 503/2013) 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. Therefore, potential adverse effects that result from combinatorial exposure of various potential stressors need specification, and their assessment needs to be prioritised. We conclude that the health risk assessment currently performed by EFSA for the maize is unacceptable. We propose testing these plants following the whole mixture approach, considering them to be "*insufficiently chemically defined to apply a component-based approach"* (EFSA, 2019).

For this purpose, EFSA should have requested the company to submit data from field trials with the highest dosage of complementary herbicides that can be tolerated by the plants, including repeated spraying. The material derived from the plants should have been assessed in regard to organ toxicity, immune responses and reproductive toxicity, also taking combinatorial effects with other plants components into account.

As a result, the toxicological assessment carried out by EFSA is not acceptable.

Allergenicity

The EFSA assessment of allergenic risks (EFSA, 2022d) is not based on a sufficiently realistic exposure to newly introduced proteins and their interactions. Different routes of exposure, the timing of exposure, microbial exposure, oral and gut microbiota composition, epithelial barrier integrity and/or non-allergenic components of the food matrix, such as immune-modulating components (adjuvants) of allergenic sources that facilitate immune responses, all have to be considered. In particular, the high number of proteins additionally expressed in the plants make it essential for appropriate data to be made available.
However, the necessary methodology was neither provided nor requested by EFSA. Therefore, the outcome of the allergenicity assessment cannot be regarded to be sufficient.

5. Environmental risk assessment

The appearance of teosinte in Spain and France (see Testbiotech, 2016; Trtikova et al., 2017) has to be considered in more detail. Maize volunteers can be found in the EU on a regular basis as has been reported from Palaudelmàs et al. (2009) in Spain or from Pascher (2016) in Austria. Further, in awareness of the biological characteristics of the GE maize and the findings of Fang et al. (2018), the maize needs to be examined in detail regarding next generation effects, volunteer potential (persistence) and gene flow. Under these circumstances, even a rare single outcrossing that goes unnoticed can have a huge long-term impact on the agro-ecosystems.

Furthermore, the EFSA (2022a) opinion is also wrong for several reasons:

- 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 have 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 genetic background that cannot be predicted from the assessment of the original event (Bauer-Panskus et al., 2020). This issue is relevant for gene flow from maize to 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 authorisation have to be regarded as not valid.

Without detailed consideration of the hazards associated with the 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.

Consequently, environmental risk assessment carried out by EFSA is not acceptable.

Testbiotech is aware of a recent EFSA statement (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, it is apparent in the updated risk assessment that EFSA still does not consider that epsps genes as such may induce fitness advantages (as noted, for example, by Fang et al., 2018; Wang et al., 2014; Yang et al., 2017). 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

For monitoring and methods to identify the specific event, Implementing Regulation 503/2013 requests:

> *The method(s) shall be specific to the transformation event (hereafter referred to as ‘event-specific’) and thus shall only be functional with the genetically modified organism or genetically modified based product considered and shall not be functional if applied to other*

transformation events already authorised; otherwise the method cannot be applied for unequivocal detection/identification/quantification. This shall be demonstrated with a selection of non-target transgenic authorised transformation events and conventional counterparts. This testing shall include closely related transformation events.

If approval for import is given, 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 GM 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.

In addition, the example of maize 87429 highlights some general problems. These are:

(1) Due to current EFSA practices it is not possible to access the original data from the companies within the period of consultation. Therefore, the opinion has to provide all the necessary data to allow other experts to conclude whether the provisions of GMO regulation (esp. 503/2013) are fulfilled. We are making this comment after our recent experiences in requesting access to documents, which in many instances took months to achieve. The Commission should advise EFSA to improve transparency.

(2) A Testbiotech report published in 2021 (Testbiotech, 2021), shows how the European Food Safety Authority (EFSA), which is responsible for risk assessment of GE plants, intentionally puts crucial issues aside. This careless approach exemplifies the overall decrease in general food safety standards that has been ongoing since the introduction of GE plants. The number of events authorised for import has, at the same time, steadily increased. In light of these findings, the Commission should try to avoid ‘rubber stamping’ all applications for import of GE plants, and thus reduce the overall number of products entering the market, while ensuring that these products undergo much more thorough risk assessment.

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