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Testbiotech comment on EFSA's assessment of genetically engineered maize MON88017 for renewal authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-014) from Bayer/Monsanto

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Introduction

The EFSA GMO panel assessed genetically engineered maize MON88017 for renewal of authorisation. This maize produces

- Cry3Bb1 protein against the larvae of *Coleoptera* (beetles) that feed below the ground;
- CP4 EPSPS protein for tolerance to glyphosate-containing herbicides.

Implementing Regulation 503/2013 was applied in the EFSA risk assessment.

1. Molecular characterisation

According to EFSA, the dossier for renewal of authorisation contained further information regarding molecular characterisation (EFSA, 2020a):

> *At the time of submission of the renewal dossier, the applicant provided a complete bioinformatic dataset for maize MON 88017 event including an analysis of the insert and flanking sequences, an analysis of the potential similarity to allergens and toxins of the newly expressed proteins and of all possible open reading frames (ORFs) within the insert and spanning the junction sites [….].*"

This dataset revealed that *"the maize endogenous gene "putative purine permease 11" has been interrupted by the insert MON 88017"* (EFSA, 2020b).

EFSA (2020a) should have requested a much more detailed investigation into potential biologically active gene products and changes in metabolic pathways. In order to assess the sequences encoding the newly expressed proteins, or any other open reading frames (ORFs) as well as interrupted genetic information present within the insert and spanning the junction sites, it was assumed that the proteins that might emerge from these DNA sequences would raise no safety issues; therefore, no detailed investigations were carried out in this regard. Furthermore, other gene products such as dsRNA from additional open reading frames were not assessed. Thus, uncertainties remain about other biologically active substances arising from the method of genetic engineering and the newly introduced gene constructs.

In regard to the expression of the additionally inserted genes, Implementing Regulation 503/2013 requests "*protein expression data, including the raw data, obtained from field trials and related to the conditions in which the crop is grown*".

Indeed, there are reasons why the data presented do not represent the conditions in which the plants are grown: (1.1) no extreme weather conditions were taken into account; (1.2) the field trials did not take current agricultural management practices into account.

1.1
The applicant did not deliver any new data regarding the expression of transgenic proteins. However, data from the initial application shows that Cry3Bb1 and CP4 EPSPS levels differed widely between field trials conducted at three different field locations in the USA during the 2002 growing season (EFSA, 2009).

> *"Across the developmental stages examined, the mean Cry3Bb1 protein levels ranged between 260-570 µg/g dw in leaf, 220-500 µg/g dw in the whole plant and 100 -370 µg/g dw in root tissues. CP4 EPSPS protein levels ranged between 150-220 µg/g dw in leaf and 70-150 µg/g dw in root. This plant material was also used to analyse the expression of the proteins in pollen, silk, forage, forage root, grain, stover and senescent roots. The mean Cry3Bb1 protein level in the grain was 15 µg/g dw (range 10-22 µg/g dw) and CP4 EPSPS protein level in grain was 5.8 µg/g dw (range 4.1-7.1 µg/g dw)."*

Environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). There is plenty of evidence that drought or heat can significantly impact the content of Bt in the plant tissue (Adamczyk & Meredith, 2004; Adamczyk et al., 2009; Chen et al., 2005; Dong & Li, 2006; Luo et al., 2008; Then & Lorch, 2008; Trtikova et al., 2015). Therefore, to assess gene expression, the plants should have been grown under conditions of severe drought, with and without irrigation, with and without application of the complementary herbicide, as well as compared to more moderately severe climate conditions. However, no such data were requested or used for detailed comparison to assess the genome x environment interactions.

Furthermore, Fang et al. (2018) showed that stress responses can lead to unexpected changes in plant metabolism inheriting additional EPSPS enzymes. Therefore, the plants should have been subjected to a much broader range of stressors to gather reliable data on gene expression and functional genetic stability.

Moreover, the plants should have been subjected to a much broader range of defined environmental conditions and stressors (for example, those which might be expected due to ongoing climate change) to gather specific and reliable data on gene expression and functional genetic stability.

1.2
Due to increased weed pressure, it has to be expected that these plants will be exposed to high and repeated doses of glyphosate. Higher dosages of the herbicide 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 ignored in the EFSA risk assessment for renewal of maize MON88017. EFSA should have requested the applicant to submit more recent data from field trials, also taking into account the highest dosage of glyphosate that can be tolerated by the plants, including repeated spraying.

However, this aspect was ignored in the EFSA risk assessment.

Additional findings

No detailed examination was undertaken regarding the extent to which the modification of the Bt protein Cry3Bb1 will change biological characteristics. In order to enable further independent risk assessment, the full DNA sequence inserted into the plants should be made available, including all open reading frames.

EFSA also did not request a detailed analysis based on so-called 'omics' (transcriptomics, metabolomics, proteomics) to investigate changes in the overall metabolism in the plants. EFSA assumed that the data from phenotypic characteristics and compositional analysis would not indicate any need for further investigations. In general, data on phenotypic characteristics and compositional analysis can be used as complementary data, but these are not as sensitive as -omics data and cannot replace them.

Further, the method used to determine the amount of Bt toxins (ELISA) is known to be dependent on the specific protocols used. The data are not sufficiently reliable without further evaluation by independent labs. For example, Shu et al. (2018) highlight difficulties in measuring the correct concentration of Bt toxins produced by the genetically engineered plants (see also Székács et al., 2011). Without fully evaluated test methods to measure the expression and the concentration of the Bt toxins, risk assessment will suffer from substantial methodological gaps. Based on such poor and inconclusive data, the dietary exposure to Bt toxins within the food chain cannot be determined as required by Regulation (EU) No 503/2013.

Conclusion on molecular characterisation

We conclude that the plants should have been subjected to a much broader range of defined stressors to gather reliable data on gene expression and functional genetic stability, taking into account more extreme drought conditions. Furthermore, EFSA should have requested the applicant to submit data from more recent field trials, also taking into account the highest dosage of the complementary herbicides that can be tolerated by the plants, including repeated spraying.

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, as well as changes in metabolic pathways and the emergence of unintended biologically active gene products. Such in-depth investigations should not depend on findings indicating potential adverse effects, they should always be necessary to come to sufficiently robust conclusions to inform the next steps in risk assessment.

2. Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)

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."*

However, no data are presented in the renewal assessment regarding currently applied agricultural practices and changes in meteorological and agronomic conditions under which the crop is to be grown. (2.1) No extreme weather conditions were taken into account; (2.2) the field trials did not take current agricultural management practices into account.

2.1
According to EFSA, no new field trial data were presented by the applicant. Data from the first assessment of maize MON88017 (EFSA, 2009) show that field trials for compositional and agronomic assessment were conducted in the US, Argentina and Europe for only one year. No information on weather conditions was published for any of these trials.
It is not acceptable that EFSA failed to require further studies and more data, e.g.

- No field trials were conducted that lasted more than one season. Thus, based on current data, it is hardly possible to assess site-specific effects.
- No data were generated representing more extreme environmental conditions, such as those caused by climate change resulting in more extreme droughts.

In addition, Fang et al. (2018) showed that stress responses can lead to unexpected changes in plant metabolism inheriting additional EPSPS enzymes. Available publications strongly indicate that plants producing additional EPSPS enzymes are likely to show strong responses in gene expression under stress conditions, such as drought. These effects are also likely to impact plant composition and biological characteristics that are crucial for the assessment of food and feed safety. However, no specific data were requested or used for detailed comparison to assess genome x environment interactions.

Therefore, the plants should have been subjected to a much broader range of defined environmental conditions and stressors to gather reliable data.

2.2
Due to high weed pressure in many maize growing regions, it has to be expected that these plants will be exposed to higher and repeated dosages of glyphosate. Therefore, it has to be taken into consideration that the plants can be repeatedly sprayed with high dosages of the herbicide. These agricultural practices have to be taken into account to assess whether the expected agricultural practices will influence the expression of the studied endpoints.

Industry recommendations suggest dosages to be sprayed on herbicide-resistant maize of up to approx. 3,5 kg a.i./ha glyphosate post-emergence, 9 kg per season, and even higher rates (www.greenbook.net/monsanto-company/roundup-weathermax; www.greenbook.net/monsanto-company/roundup-ultra). From the available data, it has to be assumed that the specific patterns of complementary herbicide applications will not only lead to a higher burden of residues in the harvest, but may also influence the composition of the plants and agronomic characteristics. This aspect, was completely ignored in the EFSA risk assessment.

EFSA should have requested the applicant to submit data from field trials, also taking into account the highest dosage of the complementary herbicides that can be tolerated by the plants, including repeated spraying. Only the application of high and repeated dosages of glyphosate should have been regarded as representative for expected agricultural practices.

Further findings

Compositional analysis assessed by EFSA in 2009 (EFSA, 2009), revealed a range of statistically significant differences in the composition of maize MON88017 and its non-GM comparator.

Therefore, EFSA should have requested further tests for the current application, for example, including repeated spraying with higher herbicide dosages and exposure to a much wider range of environmental conditions, also taking more extreme drought conditions into account. Furthermore, the plant material should have been assessed by using 'omics-techniques' to investigate changes in plant composition or agronomic characteristics in more detail.

However, according to EFSA (2020a), no further field trials and no updated compositional analysis was requested/prepared by EFSA. Therefore, questions concerning the overall safety of the whole food and feed remain unanswered.

Based on the available data, no final conclusions can be drawn on the safety of the plants. The data do not fulfill the requirements of Implementing Regulation 503/2013.

3. Toxicology

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;"*

EFSA assessed a subchronic 90-day feeding study with maize MON88017 in 2009 (EFSA, 2009). No new data regarding toxicity were delivered by the applicant for the renewal process.

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 high number of effects should have been considered as a starting point for much more detailed investigation of potential health impacts.

However, EFSA did not request any new data regarding the food and feed safety of maize MON88017, whereas the need for more detailed assessment is underlined by publications showing that the Bt toxins also raise further questions in regard to feed and food safety:

(1) There are several partially diverging theories about the exact mode of action of the Bt toxins at the molecular level (see Then, 2010; Hilbeck & Otto, 2015). Thus, it cannot be excluded a priori that the toxins are inert in regard to human and animal health as maintained under risk assessment for food and feed.

(2) There are further uncertainties regarding the specificity of Bt toxins (Venter and Bøhn, 2016). Changes in specificity may emerge from structural modifications performed to render higher efficacy. For example, the proteins are truncated to become activated (see Hilbeck & Schmidt, 2006).

(3) In addition, there are findings in mammalian species showing that Bt toxicity is a relevant topic for detailed health risk assessment: some Cry toxins are known to bind to epithelial cells in the intestines of mice (Vázquez-Padrón et al., 1999).

(4) As far as potential effects on health are concerned, several publications (Thomas and Ellar 1983; Shimada et al., 2003; Mesnage et al., 2013; Huffman et al., 2004; Bondzio et al., 2013) show that Cry proteins may indeed have an impact on the health of mammals. For example, de Souza Freire et al., (2014) confirm haematological toxicity of several Cry toxins. Some of these effects seem to occur where there are high concentrations and tend to become stronger over longer periods of time.

(5) Further, the toxicity of Bt toxins can be enhanced through interaction with other compounds, such as plant enzymes (Zhang et al., 2000, Zhu et al., 2007; Pardo-López et al., 2009); other Bt toxins (Sharma et al., 2004; Tabashnik et al., 2013; Bøhn et al. 2016, Bøhn, 2018); gut bacteria (Broderick et al., 2009); residues from spraying with herbicides (Bøhn et al., 2016, Bøhn, 2018) and other (Kramarz et al., 2007; Kramarz et al., 2009; Khalique & Ahmed, 2005; Singh et al., 2007; Zhu et al., 2005; Mason et al., 2011; Reardon et al., 2004).

In this context, it is relevant that Bt toxins can survive digestion to a much higher degree than has been assumed by EFSA. Chowdhury et al. (2003) and Walsh et al. (2011) showed that when pigs were fed with Bt maize, Cry1A proteins could frequently and successfully still be found in the pig colon at the end of the digestion process. This means that Bt toxins are not degraded quickly in the gut and can persist in larger amounts until digestion is completed; therefore, there is enough time for interaction between various food compounds.

Further, as far as the exposure of the food chain with Bt toxins is concerned, EFSA should have requested data on the overall combined exposure to Bt toxins resulting from the introduction of Bt plants in the EU. Currently, there are already 40 events that produce Bt toxins authorised for import. The accumulated exposure stemming from these imports should have been taken into account. For example, a new study testing corn with a combination of Bt toxins (Cry1Ab and Cry34Ab1) indicates health impacts in rats (Zdziarski et al., 2018).

We concluded there is a need for more detailed investigation. Further, more detailed (e.g. using several dosages) and long-term feeding studies, taking into account the functioning of the microbiome, would be necessary to assess potential health impacts. These studies should include -omics data from animals, as well as detailed assessment of the impact of higher dosages of glyphosate sprayed on the plants (as might be expected under practical conditions).

Beyond that, the residues from spraying were considered to be outside the remit of the GMO panel (EFSA, 2020b). However, without detailed assessment of these residues, no conclusion can be drawn on the safety of the imported products: due to specific agricultural practices in the cultivation of these herbicide resistant plants, there are, for example, specific patterns of applications, exposure, occurrence of specific metabolites and emergence of combinatorial effects that require special attention (see also Kleter et al., 2011).

More detailed assessment is also in accordance with pesticide regulation that requires specific risk assessment of imported plants if the usage of pesticides is different in the exporting countries compared to the usage in the EU. In this regard, it should be taken into account that EFSA (2019a) explicitly stated that no conclusion can be derived on the safety of residues from spraying with glyphosate occurring in genetically engineered plants resistant to this herbicide. Just recently, new doubts were raised about results from previous feeding studies which came to the conclusion that glyphosate-resistant maize is safe for human and animal consumption (Séralini, 2020).

There is a common understanding that commercially traded formulations of glyphosate, such as Roundup, can be more toxic than glyphosate itself. Therefore, the EU has already taken measures to remove problematic additives known as POE tallowamine from the market. Problematic additives are still allowed in those countries where the genetically engineered plants are cultivated. The EU Commission has confirmed the respective gaps in risk assessment:

> *"A significant amount of food and feed is imported into the EU from third countries. This includes food and feed produced from glyphosate-tolerant crops. Uses of glyphosate-based plant protection products in third countries are evaluated by the competent authorities in those countries against the locally prevailing regulatory framework, but not against the criteria of Regulation (EC) No. 1107/2009. (…)." (*www.testbiotech.org/content/eu-commission-request-consider-impact-glyphosate-residues-feed-animal-health-february-2016)

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

There are further relevant issues: for example, the potential impact on the intestinal microbiome also has to be considered. Such effects might be caused by the residues from spraying since glyphosate has been shown to have negative effects on the composition of the intestinal flora of cattle (Reuter et al., 2007), poultry (Shehata et al., 2013) and rodents (Mao et al., 2018). In general, antibiotic effects and other adverse health effects might occur from exposure to a diet containing these plants, which were not assessed under pesticide regulation.

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 (see also van Bruggen et al., 2017). Further attention should be paid to the specific toxicity of the metabolites in the active pesticide ingredient.

Whatever the case, both the EU pesticide regulation and the GMO regulation 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 to be a prerequisite for granting authorisation.

The maize is engineered to be glyphosate-resistant and also produces an insecticide, we therefore propose testing these plants following the whole mixture approach, considering them to be "*insufficiently chemically defined to apply a component-based approach"* (EFSA, 2019b). This approach would require to take into account whole food and feed material prepared from the maize as currently grown and imported. 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.

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 is unacceptable.

4. Allergenicity

Implementing Regulation 503/2013 requests:

> *"In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the applicant shall assess the possible role of these proteins as adjuvants. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity."*
>
> *"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;"*

EFSA opinions on MON88017 (EFSA, 2009; 2020a) contain only limited information regarding the assessment of allergenicity. According to EFSA (2009), only *in-vitro* tests were conducted to assess allergenicity of MON88017. EFSA (2020a) only mentions:

> *"At the time of submission of the renewal dossier, the applicant provided a complete bioinformatic dataset for maize MON 88017 event including an analysis of the insert and flanking sequences, an analysis of the potential similarity to allergens and toxins of the newly expressed proteins and of all possible open reading frames (ORFs) within the insert and spanning the junction sites, an analysis of possible horizontal gene transfer (EFSA, 2017b), and a safety assessment of the newly expressed proteins Cry3Bb1 and CP4 EPSPS regarding their capacity to trigger celiac disease (EFSA GMO Panel, 2017a). Upon EFSA request, the applicant provided additional information followed by further clarifications on the celiac disease analysis for Cry3Bb1 protein."*

We appreciate that EFSA took into account risks concerning celiac disease. However, there are other inflammatory processes triggered by less well-defined mechanisms and immune responses which also are relevant in this context.

Contrary to what is suggested by the findings of in-vitro studies (EFSA, 2009), Bt toxins might not be degraded quickly in the gut but are likely to occur in substantial concentrations in the large intestine and faeces (Chowdhury et al., 2003; Walsh et al., 2011).

In regard to the degradation of the Bt toxins during ingestion, there is specific cause for concern that the maize or gluten is likely to be fed together with soybeans that naturally produce enzymes, which can substantially delay the degradation of Bt toxins in the gut (Pardo-López et al., 2009). In addition, soybeans are known to produce many food allergens. Therefore, the immune system

responses caused by the allergens in the soybeans might be considerably enhanced by the adjuvant effects of the Bt toxins. Furthermore, in processed products, such as maize gluten, the toxins can even show a much higher concentration.

Furthermore, it also has to be taken into account that so far only very few Bt toxins produced in genetically engineered plants have been investigated in regard to their potential impact on the immune system. As yet, only two Bt toxins (Cry1Ac and Cry1Ab) have been tested for their possible effects on the immune system. While the applicant provided some data on Cry3Bb1 in regard to celiac disease, other diseases associated with symptoms of chronic inflammation were not considered at all.

Given the fact that potential effects of Bt toxins on the immune system have meanwhile been discussed for many years (for overview see, for example, Then & Bauer-Panskus, 2017), and already around 40 GE crops events producing Bt toxins have been approved for the EU market, any further delay in resolving these crucial questions is unacceptable. In accordance with EU Regulation 1829/2003, safety of whole food and feed has to be demonstrated before renewal of approval for import can be issued. Since this is not the case with maize MON88017, the risk assessment is not conclusive and market authorisation cannot be granted.

In summary, the EFSA assessment of maize MON88017 does not fulfill the requirements for assessing risks to the immune system.

5. Others

(1) 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 re-approval for import is granted, the applicant has to ensure that post-market monitoring (PMM) is developed to collect reliable information to detect indications of any (adverse) effects on health that 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 (see also comments from Member States experts, EFSA, 2020b).

(2) We agree with comments made by experts from Member States (EFSA, 2020b), that the applicant should be asked to provide a detailed analysis of the fate of the Bt proteins in the environment and a quantitative estimate of subsequent exposure of non-target organisms.

Besides methods of detection, other methods for quantifying exposure to the insecticidal proteins need to be made publicly available in order to facilitate monitoring. Food and feed producers, farmers as well as experts dealing with environmental exposure (for example, which waste material, spillage and manure) have to be able to gather independent information on their exposure to the toxins via independent laboratories. As yet, these methods are regarded as confidential business information and are not made available upon request by EFSA. Thus, the Commission should ensure that the relevant data are both publicly available and reliable.

As existing evidence shows (Székács et al., 2011; Shu et al., 2018), the methods need to be carefully evaluated to ensure that the results are reliable, comparable and reproducible. Therefore, fully evaluated methods have to be published that allow the Bt concentration in the maize to be measured by independent scientists, as is the case for other plant protection compounds used in food and feed production. This is necessary to make sure that the environment as well as human and animals coming into contact with the material (for example, via dust, consumption or manure) are not exposed to higher quantities of Bt toxins than described in the application.

(3) It should be noted that EFSA communication with Member States is not always adequate. In its responses to concerns of MS experts, EFSA often seems to use copy-paste texts not related to the renewal application of maize MON88017, but to other applications (EFSA, 2020b). In several places, false EFSA question numbers are given or wrong GM events mentioned. Amongst others, examples include:

- "*… the applicant performed a literature search in the context of application EFSA* ***GMO-RX-016****.*" [correct: GMO-RX-014]
- *"The GMO Panel acknowledged that no scientific publications raising a safety concern for human and animal health and the environment which would change the original risk assessment conclusions on maize* **MIR604** *had been identified by the applicant."*
- *"Moreover, in its scientific opinion on application EFSA-****GMO-RX-016****, the GMO Panel concluded that no new hazards or modified exposure and no new scientific uncertainties were identified for the application for renewal that would change the conclusions of the original risk assessment on maize MON 88017."*

Member States might get the impression that EFSA does not take their comments and the authorisation process seriously.

6. Environmental risk assessment

No updated environmental risk assessment of MON88017 was conducted by EFSA (2020a). However, the appearance of teosinte in Spain and France (see Testbiotech, 2016; Trtikova et al., 2017) should be considered in detail. As Pascher (2016) shows, the volunteer potential of maize is higher than previously assumed. Further, in awareness of the biological characteristics of the 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.

Without 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. 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 (Kawata et al., 2009; Cao et al., 2009;

Yang et al., 2017; Bollinedi et al., 2017; Lu & Yang, 2009; Vacher et al., 2004; Adamczyk & Meredith, 2004; Adamczyk et al., 2009). This issue is relevant for gene flow from maize to teosinte as well from teosinte to maize.

EFSA should have requested new 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 invalid.

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.

7. Conclusions and recommendations

EFSA risk assessment cannot be accepted.

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