

## TESTBIOTECH Background 8 - 12 - 2019

### Testbiotech comment on EFSA's assessment of genetically engineered maize MON87427 x MON89034 x MIR162 x MON87411 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2017-144) from Bayer/Monsanto

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#### Introduction

The EFSA GMO panel assessed the four-stacked maize MON87427 x MON89034 x MIR162 x MON87411, which is derived from crossing genetically engineered maize events (EFSA, 2019f). The parental plants were assessed by EFSA in previous opinions. The maize contains genes conferring doubled resistance to glyphosate and produces three insecticides:

- MON87427 expressing CP4 EPSPS protein for tolerance to glyphosate-containing herbicides;
- MON89034 expressing the insecticidal proteins Cry1A.105 (artificially synthesized) and Cry2Ab2;
- MIR162 expressing the insecticidal protein Vip3Aa20 and phosphomannose isomerase (PMI) which is a selectable marker;
- MON87411 produces an insecticidal dsRNA (DvSnf7 dsRNA) as well as the EPSPS protein for tolerance to glyphosate-containing herbicides. In addition it also produces the insecticidal protein Cry3Bb1 (artificially synthesized).

Consequently, the stacked GE maize has doubled resistance to glyphosate, making it tolerant to high dosages and repeated sprayings as applied in fields with herbicide-resistant weeds. Further, it produces three toxins against the larvae of *Lepidoptera* (butterflies) that feed on the plants ('pest insects') and one toxin (Cry3Bb1) against the larvae of *Coleoptera* (beetles) that feed below the ground. In addition, it produces an biological active molecule (dsRNA) that can negatively interfere with metabolism in larvae of *Coleoptera* (beetle). In addition, it produces the PMI protein (phosphomannose isomerase) derived from *Escherichia coli*. Expression of PMI enables transformed maize cells to utilise mannose and therefore to survive on specific media used for selecting the maize plants after the process of genetic engineering (so called marker gene).

Implementing Regulation 503/2003 has been applied in the risk assessment as performed by EFSA.

#### 1. Molecular characterisation

The process of genetic engineering involved several deletions and insertions in the parental GE maize plants. In order to assess the sequences encoding the newly expressed proteins or any other open reading frames (ORFs) 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.

Previous research has indicated that expression of Cry1A.105, Cry2Ab2 and EPSPS proteins in genetically engineered maize can induce changes in the overall proteome of the respective GE maize line, with impacts on associated endogenous metabolic pathways (Agapito-Tenfen et al., 2014). These transgenes are also present in the stacked maize. Thus, robust data should have been presented to assess whether metabolic changes with relevance to biosafety occur in the stacked maize.

Therefore, EFSA (2019f) should have requested much more detailed investigation into potential biologically active gene products and changes in metabolic pathways.

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*”.

However, there are three reasons why the data presented do not represent the conditions in which the plants are grown: (1.1) the field trials were not conducted in all relevant regions where the maize will be cultivated, and no extreme weather conditions were taken into account; (1.2) the field trials did not take current agricultural management practices into account; (1.3.) only one transgenic variety was included in the field trials.

#### 1.1

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 and in comparison 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. However, the expression of the additional enzymes was only measured under field conditions in the US for one year. 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. Whatever the case, they should have been tested in maize producing countries in South America.

In consequence, the available publications strongly indicate that plants inheriting EPSPS enzymes are likely to show strong reactions in their gene expression when grown under stress conditions, such as drought. These effects are also likely to impact plant composition and biological characteristics crucial for the assessment of food and feed safety. However, no specific data were requested or used for detailed comparison to assess the genome x environment interactions.

Whatever the case, the plants should have been subjected to a much broader range of defined environmental conditions and stressors (which for example have to be expected under ongoing climate

change) to gather 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 also repeated dosages of glyphosate. Higher applications 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 completely ignored in the EFSA risk assessment. EFSA should have requested the applicant to submit data from field trials with the highest dosage of glyphosate that can be tolerated by the plants, including repeated spraying.

As mentioned by the experts of Member States, application of higher rates the complementary herbicides can cause stress reactions in the plants and impact gene expression (EFSA, 2019d). However, this aspect was ignored in the EFSA risk assessment.

### 1.3

It is known that the genomic background of the variety can influence the expression of the inserted genes (see, for example, Trtikova et al., 2015). Therefore, EFSA, should have requested additional data from several varieties, including those cultivated in South America.

#### Additional findings

The findings (1.1 – 1.3) on flaws in risk assessment are supported by data from previous applications with the same parental events. Data presented in Table 1 show widely differing gene expression and content of Vip3Aa20.

Table 1: Gene expression and content of Vip3Aa20 present in maize MIR162 in grain ( $\mu\text{g/g}$  dry weight, mean values)

Application (EFSA opinion)	Details from field trials	Content of Vip3Aa20
MON87427 x MON89034 x MIR162 x MON87411 (EFSA 2019f)	Field trials at five locations in the USA in 2014 (sprayed with glyphosate)	52
MON87427 x MON87460 x MON89034 x MIR162 x NK603 (EFSA 2019a)	Field trials at five locations in the USA in 2014 (sprayed with glyphosate)	38
MON87427 x MON89034 x MIR162 x NK603 (EFSA 2019b)	Field trials at five locations in the USA in 2013 (sprayed with glyphosate)	59
Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 (EFSA 2019c)	Field trials at three locations in the US in 2012 (not sprayed with complementary herbicides)	100
Bt11 x MIR162 x 1507 x GA21 (EFSA 2018a)	Field trials at one single location in the US 2008 (sprayed?)	28
Bt11 x MIR162 x MIR604 x GA21 (EFSA 2015a)	Single location in the US in 2006 (sprayed?)	140
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

These data show a range of mean values between 28  $\mu\text{g/g}$  and 140  $\mu\text{g/g}$  for Vip3Aa20 in the grain, while in other cases even 166  $\mu\text{g/g}$  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.

The factors influencing the content might seem variable. As EFSA (2012) stated in previous opinions “*a year-to-year and site-to-site variation is evident*”. In addition, genetic backgrounds of different varieties and effects from stacking seem to be relevant as well. There is no justification for not requesting additional data on the impact of drought conditions on Vip3Aa20 gene expression.

In general, EFSA fails to give a full overview of existing data from previous applications and findings to facilitate an examination of the range of gene expression in more detail, and to derive a conclusive and sufficiently robust risk assessment.

### Further findings

dsRNA can have many functions and interact with gene regulation in many ways. In most cases, gene activity will be blocked or down regulated (silenced). In many cases, there may be cross-kingdom activity. They are known to interact with gene regulation in microorganisms, insects, plants and mammals. Its specificity is dependent on several factors such as its stability, further splicing and regions within DNA where it can interact.

To assess potential off-target effects, the structure of the dsRNA can be compared with genomic regions in organisms that might come into contact with the molecules. Regulation (EU) No 503/2013 says that when silencing approaches with RNAi are used in genetically engineered plants, a bioinformatics analysis is required in order to identify potential 'off-target' genes. An important starting point is the collection of relevant data to make comparisons with the RNA networks of non-target organisms, including mammals and humans that are exposed to the plants via food and feed.

However, in the assessment of the parental plant MON87411 (EFSA, 2018b), the additional dsRNA produced in the plants was compared only with RNA as expressed in plants. EFSA concluded that there was similarity that would raise concerns. However, no comparison was made in regard to mammals and microorganisms.

This gap in risk assessment was also expressed in comments from the experts of Member States (EFSA, 2018c), such as the BVL (Germany):

*“The applicant has not provided data on potential RNAi-targets of DvSnf7 dsRNA in non-target organisms, including humans. (...) Thus, additional data like bioinformatic evaluations should be considered. As demonstrated by a history of safe consumption of dsRNAs with high homology in conventional food and feed, the identification of sequence similarities between the dsRNA produced by MON 87411 and transcripts of exposed species would not directly indicate an increased risk of adverse effects. Nevertheless, a bioinformatic search for potential targets in transcripts of human and likely exposed non target species (farm animals) would back the weight of evidence approach if no matching targets were identified. The German Competent Authority therefore recommends a bioinformatic evaluation, comparable to study no.: RAR-2015-0373, to identify potential target genes in human and other relevant non target species. Additional information might be recommended according to the outcome of the bioinformatics evaluation.”*

However, no such data were requested by EFSA for the parental plants nor the stacked events. Instead, EFSA seems to be of the opinion that such data would not allow reliable prediction of the potential effects of such molecules. The protocol of the EFSA panel meeting (EFSA, 2017) states:

*“In plants a set of parameters allows for a reasonable prediction of RNAi off-target genes while in human and animals the extent of complementarity between the small RNA and the target is more limited and therefore these prediction tools do not allow for sufficiently reliable predictions (Pinzón et al., 2017). Therefore the GMO Panel considers that only the search for small RNA off-targets in the GM plant could have value for the risk assessment of GM plants.”*

This is an interesting statement since it exposes some limitations in current knowledge. Pinzón et al. (2017) show that further research would be needed to make reliable predictions in regard to dsRNA effects in mammals. This publication can not be used as justification not to assess health risk in the case of MON87411. But EFSA neither tries to overcome these limitations of current knowledge, nor does it consider that risk assessment cannot be concluded without sufficient data and meaningful analysis.

Instead, EFSA (2018b) simply accepts these limitations by restricting its considerations and risk assessment to potential off-target effects in the plants, leaving aside effects in humans and livestock and their gut microbiomes that are exposed to the maize via the food and feed chain. This is akin to someone who has lost something in the dark and then only searches where street lamps shed light because that is where the light is available.

A similar approach was taken by EFSA in assessing the concentration of dsRNA and its downstream metabolic products in the plants. EFSA (2018b) states:

*“The applicant provided a measure of the levels of DvSnf7 dsRNA in different tissues including grain and forage. However, the dsRNA is an intermediate molecule which is processed by dicer to siRNA molecules and the levels of dsRNA are not a good proxy for the levels of the active siRNAs in the plant (Paces et al., 2017). Therefore, the levels of the DvSnf7 dsRNA were not considered relevant for the risk assessment of maize MON 87411.”*

As a result, the data on the concentration of the biologically active molecules in the plants were not assessed. However, such data are necessary to assess the risks for the food chain and the fate of these molecules in the environment (see below).

Instead of performing detailed risk assessment, EFSA (2018b), in contradiction to scientific publications (see below) simply assumes that:

*“the amount of RNAs taken up and absorbed after oral ingestion is considered negligible in humans and animals (mammals, birds and fish).”*

EFSA’s risk assessment of the dsRNA expressed in the plants on a molecular level might be described as the perfect example of a ‘don’t look – don’t find’ strategy incompatible with existing regulation.

There are further gaps in risk assessment:

EFSA did not assess additional unintended gene products, such as other unintended dsRNA, that can emerge from the insertion of the transgenes.

Further, no detailed consideration 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 any 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. However, these data did show many significant changes (see below). 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.

Expression data were provided on the new intended proteins. It is known that the Bt content in the plants depends on environmental impact. For example, environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). Therefore, the plants should have been subjected to a much broader range of defined environmental conditions and stressors in order to gather reliable data on gene expression and functional genetic stability. The same investigations should be performed in regard to dsRNA produced in the maize.

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 and the dsRNA (and its metabolites), 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. A similar problem emerges from the dsRNA produced in the plants.

Consequently, the risk assessment of molecular characteristics is not conclusive and is not sufficient to show food and feed safety.

### Conclusion on molecular characterisation

We conclude that the available data strongly indicate gene expression of several of the newly introduced genes is likely to depend on, or be influenced by, stacking, varietal background, the spraying of the herbicide or environmental conditions such as drought.

Therefore, 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, taking into account more extreme drought conditions. In addition, they should have been tested in the maize producing countries in South America. Furthermore, EFSA should have requested the applicant to submit data from field trials with the highest dosage of the complementary herbicides that can be tolerated by the plants, including repeated spraying. In addition, EFSA should have requested data from several varieties, including those cultivated in South America.

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 plants genome, as well as changes in metabolic pathways and the emergence of unintended biological 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.

Finally, it is not acceptable that the molecular characterisation of the dsRNA as produced in the plants does not allow an assessment of its non-target across kingdom effects and the concentration of the toxin the plants can not be determined.

## **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, the data presented do not represent expected agricultural practices or the different meteorological and agronomic conditions under which the crop is to be grown. There are three reasons: (2.1) the field trials were not conducted in all relevant regions where the maize will be cultivated, and no extreme weather conditions were taken into account; (2.2) the field trials did not take the current agricultural management practices into account; (2.3) only one transgenic variety was included in the field trials.

## 2.1

Field trials for compositional and agronomic assessment of the stacked maize were conducted in the US for only one year and not in other relevant maize production areas, such as Brazil and Argentina. As shown in the EFSA opinion (2019f), *“no exceptional weather conditions were reported at any of the selected field trial sites”*. In addition, and contrary to the expected agricultural practices or the different meteorological and agronomic conditions under which the crop is to be grown, EFSA states it *“considers that the selected sites reflect commercial maize-growing regions in which the test materials are likely to be grown.”*

Taking into account the purpose of the genetic engineering in this case, it is not acceptable that EFSA failed to require further studies 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. However, as our analysis on gene expression shows, specific site by site and year by year effects have to be expected.
- No data were generated representing more extreme environmental conditions, such as those caused by climate change resulting in more extreme droughts.
- No data were generated that represent the growing conditions in other relevant maize growing regions outside the US.

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 reactions 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 amounts and repeated dosages of glyphosate. It has to be taken into account that the herbicides can be sprayed with high dosages and repeated sprayings. These agricultural practices have to be taken into account to assess whether the expected agricultural practices will influence the expression of the studied endpoints. However, this requirement was mostly ignored by EFSA and the company: glyphosate was only sprayed at an early stage of vegetation and at comparably low dosages.



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](http://www.greenbook.net/monsanto-company/roundup-weathermax); [www.greenbook.net/monsanto-company/roundup-ultra](http://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, which is supported by the analysis of the gene expression provided above, was ignored in the EFSA risk assessment.

EFSA should have requested the company to submit data from field trials with the highest dosage of the complementary herbicides that can be tolerated by the plants, including repeated spraying with each active ingredient individually as well as in combination. Taking into account the specific characteristics of the stacked maize, only the application of high and repeated dosages of glyphosate should have been regarded as representative for expected agricultural practices.

### 2.3

It is known that the genomic background of the variety can influence the expression of the inserted genes (see, for example, Trtikova et al., 2015). Therefore, EFSA should have requested additional data from several varieties, including those cultivated in South America, to examine how the gene constructs interact with the genetic background of the plants. This approach is supported by the analysis of the gene expression provided above but was ignored in the EFSA risk assessment.

#### Further findings

Only data from a low number of agronomic parameters (10) were subjected to statistical analysis in accordance with EFSA guidance, 5 (without and without spraying of the complementary herbicide) of these were found to be statistically and significantly different.

Compositional analysis of 54 endpoints in the grains revealed many (and partly major) statistically significant differences: 32 endpoints were statistically significantly different in plants sprayed with the complementary herbicides, 42 in plants not sprayed with glyphosate (but other conventional herbicides).

Even if changes taken as isolated data might not directly raise safety concerns, the overall high number of significant effects has to be taken as a starting point for much more detailed investigations: half of the parameters measured in regard to agronomic characteristics and more than half concerning plant composition were significantly different.

As explained above, EFSA should have requested further tests with repeated spraying with higher herbicide dosages and exposure to a much wider range of environmental conditions, 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, instead of assessing the overall pattern of changes in plant components, their causes and possible impacts in more detail, EFSA only assessed the observed changes in isolation in regard to evidence of potential harm. This approach turns the comparative approach into a trivial concept of assessing bits and pieces, and it ignores questions concerning the overall safety of the whole food and feed. However, more in-depth investigations should not depend on findings indicating adverse effects, they should always be necessary to come to sufficiently robust conclusions to inform the next steps in risk assessment.

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.

## **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;”*

As explained above, many significant changes were identified: half of the parameters measured in regard to agronomic characteristics and more than half of plant composition were significantly different. 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 their potential health impacts.

Despite these findings, and in awareness of the lack of more specific data and the resulting major uncertainties (such as combinatorial effects, the effects caused by the dsRNA and the artificially synthesized Bt proteins), no testing of the whole stacked plant (feeding study) was requested.

In more detail, many uncertainties are surrounding the risk assessment of the parental plant MON87411: For the single plant, the company conducted a 90-day feeding trial with maize MON87411 in rats. In this feeding trial only one dosage of maize (33 %) was included as part of the diet, instead of several dosages as requested by existing guidance. Nevertheless, EFSA still accepted the data.

The stability of the test and control materials was not tested; therefore it remains unclear if the diet is comparable to diets fed under practical conditions if, for example, the maize is fed to animals closer to the date of harvest.

The most relevant finding was weight depression in the rats fed with the maize. As EFSA (2018b) summarises :

*“Statistically significant lower mean feed consumption (as g/cage per day only) were observed in males fed test diet (~ 9% in study week intervals 5–6, 9–10, 10–11, 11–12). This was associated with a statistically significant decrease in mean body weights, compared to the concurrent control (~ 7% in weeks 11 and 12) and in mean cumulative body weight (~ 12% in study week intervals 0–10, 0–11 and 0–12). Moreover, statistically significant lower mean weekly body weight change was also observed in males (study week intervals 0–1, 3–4, and 6–7) and in females (study week interval 7–8) fed the test diet, compared to the concurrent controls.”*

However, in the absence of test diet-related clinical signs and histopathological changes in the digestive tract, the GMO panel considered the changes to be non-adverse. Further, EFSA (2018b), without citing specific references, very generally questions whether the uptake of the dsRNA can be expected at all:

*“Dietary ncRNAs [non coding RNAs] are generally rapidly denaturated, depurinated and degraded shortly after ingestion due to enzymes and conditions (e.g. pH) in the gastrointestinal tract lumen; in addition, the presence of barriers (e.g. mucus, cellular membranes) limits the cellular uptake of ncRNAs by gastrointestinal cells, and a rapid intracellular degradation of possible uptaken ncRNA occurs. Due to the above, the amount of RNAs taken up and absorbed after oral ingestion is considered negligible in humans and animals (mammals, birds and fish).”*

This assessment of toxicology has to be rejected for several reasons:

- In 2012, it was reported for the first time that miRNA produced by plants can enter the bloodstream of mammals (including humans) at the stage of consumption (Zhang et al., 2012). These findings were called into question by several experts (see, for example, US EPA, 2014; EFSA, 2014). However, looking at more recent publications, one has to assume that plant miRNA can indeed enter the bloodstream, organs, milk and urine of mammals after ingestion (Yang et al., 2015; Liang et al., 2015; Hirschi et al., 2015, Lukaski & Zielenkiewicz, 2014).
- There is evidence that small RNAs taken up from the intestine do indeed interfere with gene regulation in humans and animals. For example, it was found that miRNA transferred via milk shows biological activity (Baier et al., 2014). Small RNAs produced by plants are able to interfere with the immune system in humans and animals (Zhou et al., 2015; Cavalieri et al., 2016).
- It is also known from several studies that uptake of miRNA from the mammalian gut and its detection is dependent on specific factors. For example, Liang et al. (2015) describe mechanisms for uptake and measurement that need to be taken into account to successfully quantify the uptake, Yang et al. (2015) as well as Wang et al. (2012) show that the health status of the recipient can be decisive; Baier et al. (2014) show that packaging in liposomes enhances uptake; Yang et al. (2015) show that dosage and also prolonged duration of exposure is important.

None of these issues were discussed or assessed by EFSA (2018b). Further, an external study commissioned by EFSA (Paces et al., 2017) overlooked several relevant studies. Moreover, in its conclusions it does not support the position of EFSA that uptake cannot generally be expected. Paces et al. (2017) summarise the discussion as follows:

*“Thus, it is apparent that four years after the original report (Zhang et al., 2012(...)), the field remains split. The essential questions concerning the existence of the proposed mechanism emerged already in 2012. Further research is necessary to clarify the basis of the aforementioned contradictory observations.”*

Paces et al. (2017) also mention that the findings (Zhang et al., 2012), which although disputed are not in contradiction to the general findings in this field:

*“In 2012, the article by Zhang et al. proposed that miRNAs from ingested plants could traverse into the bloodstream and suppress genes in the liver (Zhang et al., 2012 (...)). The report sparked an ongoing debate because of potential implications these data could have. It should be pointed out that, while the article reported unexpected and surprising results, it was not breaking any conceptual dogma. The idea that information could be transmitted*

*from food in a form of a large organic molecule that would traverse into the human organism has been an integral part of the prion hypothesis, which brought a concept of food-borne infectious particles made only of proteins (...). The prion hypothesis, for which Stanley Prusiner received a Nobel Prize in 1997, is nowadays a biology textbook knowledge. Furthermore, cross-kingdom regulation by small RNAs was discovered in RNA silencing field already in its early years – long dsRNA expressed in bacteria could induce repression of worm genes with complementary sequences when worms were fed with such bacteria (...). Furthermore, in 2012 it was already well known that feeding on a plant carrying an RNAi-inducing transgene can induce RNAi in nematodes, insects, or fungi (...). Thus, the article by Zhang et al. was not bringing any major shift in existing paradigms. The article essentially extended knowledge of RNA silencing spreading by reporting an example of a miRNA activity transferred from plants to mammals through feeding.”*

There are at least two ways in which the additional dsRNA expressed in the plants can impact mammalian health:

(1) Uptake from the gut into the bloodstream in the same way as other plant miRNAs as described (see, for example, Yang et al., 2015; Liang et al., 2015; Hirschi et al., 2015; Beatty et al., 2014). If the bioactive molecules produced in the plants start to interfere with mammalian gene regulation, the effects might be drastic: in humans dysfunction of the ESCRT complex is associated with numerous pathologies, including cancer and various neurodegenerative diseases (Henne et al., 2012).

Based on current knowledge, this scenario cannot be excluded. This is especially true in the light of the specific circumstances described by Liang et al. (2014), Zhang et al. (2012) and Yang (2015) that are relevant for the uptake of miRNA from the gut. The need for further investigation is supported by the outcome of a FIFRA scientific panel workshop held in the US in 2014, maintaining that in particular the risks for immune-compromised individuals should be tested (US EPA, 2014):

*“The stability of dsRNA should be tested in individuals that manifest specific diseases (e.g., Crohn’s, colitis, irritable bowel syndrome, etc.), the immune compromised, elderly, as well as children. These individuals may have compromised digestion or increased sensitivity to dsRNA exposure.”*

(2) It is well known that miRNA plays a key role in gene regulation in the gut microbiome, as well as in the communication between the mammalian host and its gut microbiome (see, for example, Williams et al., 2017). It is plausible that the dsRNA produced in maize MON87411 can interact with the gut microbiome directly without direct uptake from the gut. At least for yeast, the essential role of the Snf7 as part of the ESCRT pathway is well described (see [www.yeastgenome.org/locus/S000004015](http://www.yeastgenome.org/locus/S000004015)). Thus, there is a plausible hypothesis on how the additional dsRNA might affect the gut microbiome community.

Interaction with the microbiome also might explain the findings from animal feeding studies showing weight differences without pathological effects.

These aspects were mostly overlooked by EFSA (2018b) in its risk assessment even though a 2014 EFSA workshop (EFSA, 2014) identified the following issues as relevant for risk assessment of health effects:

“Throughout the different discussion topics, the following issues were identified as knowledge gaps, where more research could be warranted:

- The RNAi and metabolic profiling in RNAi-based plants could be further explored and corroborated to support risk assessment. In this context, ‘omics’ techniques should be further investigated as supporting tools.
- The use of bioinformatics to predict potential off target effects in consumers should be further explored.
- Possible changes in microbiota, residing in human or animal guts, following consumption of food and feed products derived from RNAi-based plants could be a research topic.”

As the BSE crisis showed, the risk of bioactive compounds being transmitted via the food and feed chain poses a high risk for farm animals and humans (see Paces et al., 2017). Therefore, uncertainties and knowledge gaps identified in the current risk assessment cannot be accepted.

In addition, 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; Khaliq & 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 colon of pigs 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; and that 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 conclude the 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 can be expected under practical conditions).

In any case, the toxicological assessment carried out by EFSA (2018b and 2019f) is not sufficient to show food and feed safety.

Beyond that, 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 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 (2019g) 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.

Further, 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](http://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 the complementary herbicides 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 of the pesticide active ingredients that might occur specifically in the stacked event.

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.

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 stacked 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, 2019e).

Despite all these open questions regarding potential health impacts, we are not aware of a single sub-chronic or chronic feeding study performed with whole food and feed derived from the stacked maize. This observation is supported by the literature review carried out by the company which did not yield any peer reviewed publication. In this context, it is relevant to consider that the outcome of the feeding studies with the parental plants raised several questions concerning their results, methodology and reliability (see comments from the experts of Member States, EFSA, 2019d).

Testbiotech is also aware that feeding studies with similar stacked maize indicated potential health impacts such as inflammatory responses in the stomach (Zdziarski et al., 2018). Inflammatory responses are an alarm signal typical of many chronic diseases and therefore require close attention. While the applicant provided some data in regard to celiac disease, other diseases associated with symptoms of chronic inflammation were not considered at all.

In conclusion, the EFSA opinion on the application for authorisation of the stacked maize (EFSA 2019f) cannot be said to fulfil the requirements for assessment of potential synergistic or antagonistic effects resulting from the combination of the transformation events in regard to toxicology.

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

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;”*

However, EFSA did not request the applicant to provide data to verify whether the source of the transgene is allergenic. According to Santos-Vigil et al (2018), the Bt toxin Cry1Ac can act as an allergen if ingested. This publication is highly relevant: the Bt toxin Cry1Ac was used as a source for the synthesis of Cry1A.105 expressed in the stacked maize. Therefore, the synthetically derived Cry1A.105 toxin produced in the maize has structural similarity with Cry1Ac. If Cry1Ac is suspected of being an allergen, the source of Cry1A.105 has to be verified as allergenic and therefore investigated in detail.

The EU Commission initially noted that the Santos-Vigil et al (2018) publication was relevant for the risk assessment of genetically engineered plants producing Bt toxins, and therefore requested the European Food Safety Authority (EFSA) for an assessment. However, EFSA (2018d) came to the conclusion that the Santos-Vigil et al. (2018) publication does not provide any new information and suffers from methodological flaws. However, this EFSA opinion is based on a rather biased interpretation of existing publications, and it does not provide any evidence that the Santos-Vigil (2018) findings are invalid or irrelevant (Moreno-Fierros et al., 2018).

In conclusion, the EFSA assessment of the stacked maize cannot be said to fulfil the requirements for assessing allergenicity of the source of the transgene. The Santos-Vigil et al. (2018) publication has to be considered valid and not properly assessed by EFSA (Moreno-Fierros et al., 2018). In awareness of the high concentrations of insecticidal proteins produced in the stacked maize and products derived thereof, EFSA should have started with the hypothesis that the consumption of products derived from the maize can trigger allergic reactions – and should therefore have requested empirical investigations.

Furthermore, there are several studies indicating that immune responses such as adjuvanticity in mammals are triggered by Bt toxins and have to be considered in this context. Studies with the Cry1Ac toxin (Moreno-Fierros et al., 2000; Vázquez et al., 1999; Legorreta-Herrera et al., 2010; Jarillo-Luna et al., 2008; González-González et al., 2015; Ibarra-Moreno et al., 2014; Guerrero et al., 2007; Guerrero et al., 2004; Moreno-Fierros et al., 2013; Rubio-Infante et al., 2018) are especially relevant (for review also see Rubio-Infante et al., 2016).

All the responses described in the above publications are likely to be dependent on the dosage to which the mammals were exposed. In this regard, and again as mentioned above, the investigation of potential immune responses triggered by the maize is highly relevant, it has to be considered that the concentration of the insecticidal proteins is much higher in gluten meal produced from the maize, and that it can reach a much higher concentrations compared to the kernels. Therefore, the



food and feed products derived from the stacked maize need to be much more carefully risk assessed in regard to their impact on the immune system and potential adjuvanticity compared to those genetically engineered plants producing just one Bt toxin.

In its risk assessment, EFSA did not consider that under real conditions and contrary to what is suggested by the findings of in-vitro studies, Bt toxins will 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.

Our findings on gene expression show that no reliable conclusion on the content of insecticidal proteins can be derived from the available data. Furthermore, in processed products, such as maize gluten, the toxins can even show a much higher concentration. These higher overall concentrations of the three insecticidal proteins is relevant for the assessment of overall toxicology as well as for the immune system; nevertheless, there were no empirical investigations. This is especially relevant for Vip3Aa20, which so far was not subjected to more detailed analysis regarding immunological or other toxicological effects, and that can be present in comparably high concentrations in the grain.

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; none of the toxins produced in the maize were investigated in this regard in empirical research. The effects caused by a combination of these toxins also remain untested. The need for more detailed investigations in regard to potential immunogenic effects is further underlined in the minority opinion in another EFSA opinion (Annex II of EFSA, 2018a). While the applicant provided some data in regard to celiac disease, other diseases associated with symptoms of chronic inflammation were not considered at all.

In their answers to experts from Member States (EFSA, 2019d), EFSA admits only that “limited experimental evidence” is available to conclude the safety of Bt toxins in regard to immune system reactions.

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 cannot be accepted. In accordance with EU Regulation 1829/2003, safety of whole food and feed has to be demonstrated before approval for import can be issued. Since this is not the case with the stacked maize, the risk assessment is not conclusive and no market authorisation can be granted.

In summary, the EFSA assessment of the stacked maize cannot be said to fulfill the requirements for assessing risks to the immune system.

## Others

(1) From studying the statements of the experts from Member States (2019d), we have the impression that EFSA is not aware of more recent publications showing a higher degree of horizontal gene transfer (HGT) than previously thought. Further, in their interpretation of the data, EFSA seems to be adopting a biased approach based on the assumption that no HGT should be expected.

In addition, given the fact that stacked events always show a higher overall amount of additionally inserted DNA, the statistical expectation of HGT involving this specific DNA needs more consideration. We conclude that the EFSA conclusions in regard to HGT to the intestinal gut of livestock and humans as well as the fate of the DNA in the environment will need further assessment.

(2) 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.*

However, no such method for identification was made available. Based on the information available, it will not be possible to distinguish the stacked event from a mixture of single parental events or stacked events that overlap with the actual stack.

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 (see also comments from Member States experts, EFSA, 2019d).

(3) We agree with comments made by experts from Member States (EFSA, 2019d), 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 also 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.

(4) Finally, in regard to the literature research, we do not agree with the way it was carried out. The review should take into account all publications on the parental plants and provide all relevant information regarding gene expression, findings from field trials and feeding studies. Further, monitoring data should be provided on imports of parental plants into the EU.

### **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. In its assessment of the volunteer potential, the information provided by Monsanto is largely outdated. As Pascher et al. (2016) show, the volunteer potential of maize is higher than assumed by Monsanto. Further, in awareness of the biological characteristics of the maize and the findings of Fang et al. (2018), the stacked maize needs to be examined in detail regarding next generation effects, volunteer potential (persistence) and gene flow.

Furthermore, in the EFSA (2019f) 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. 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 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 stacked maize.

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

### **Conclusions and recommendations**

The EFSA risk assessment cannot be accepted.

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