# Maize DP202216 × NK603 × DAS-40278-9

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**Country: Germany**
**Type: Non Profit Organisation**

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**Comments:**

Introduction

The GMO Panel assessed maize DP202216 × NK603 × DAS-40278-9 (EFSA, 2025a). The parental plants combined in the stacked are:

• DP202216 expressing the ZMM28 protein providing the potential for yield enhancement (YE) under field conditions, as well as the PAT protein which confers tolerance to glufosinate ammonium-based herbicides,

• NK603 expressing the CP4 EPSPS and CP4 EPSPS L214P proteins which confer tolerance to glyphosate-containing herbicides,

• DAS-40278–9 expressing the AAD-1 protein which degrades 2,4- dichlorophenoxyacetic acid (2,4-D) and AOPP class of herbicides.

1. Systematic literature review

A systematic review as requested in Regulation (EU) No 503/2013 was not provided by the applicant. Despite the fact that there are many publications on the parental plants, EFSA agreed that it would not be necessary to deliver a systematic review. This practise of EFSA undermines the legally required standards according the Implementing Regulation 503/2013.

2. Molecular characterisation

The current practice of EFSA fails to fulfill the legally requested standards as requested by EU Regulation 1829/2003. According to the Regulation, it is requested that food or feed derived from genetically modified organisms (“GMOs”) must not have adverse effects on human health, animal health or the environment (Article 4(1)(a) and 16(1)(a) of the Regulation 1829/2003). As requested in Recital 10 of the Regulation: “Thus, genetically modified food and feed should only be authorised for placing on the Community market after a scientific evaluation of the highest possible standard, to be undertaken under the responsibility of the European Food Safety Authority (Authority), of any risks which they present for human and animal health and, as the case may be, for the environment.”

In the case of the present application

• only few data were assessed in regard to biologically active molecules that may emerge from open reading frames caused by the insertion of transgenes (taking into account only proteins);

• limited data on gene expression were assessed that stem from field trials not representing the full range of climatic and regional conditions under which these plants will be grown;

• data from only one genetically engineered variety were expanded to all varieties in which these constructs may be integrated.

Data from only one year (2021) from field trial sites in the US and Canada are insufficient to represent the range of conditions under which these plants will be grown. Since climate change is ongoing in regions where the plants are cultivated, the data from the applicant should allow to extrapolate for a period of 10 years (the duration of the aimed market approval). Therefore, the data provided are invalid and do not allow any conclusion in regard to risk assessment.

In regard to the intended enhanced yield potential provided by DP202216, field trials showed no higher yields for maize DP202216 × NK603 × DAS-40278-9. An absence of the yield gain was already shown in the data for the parental plant (EFSA, 2024). Only observing an increased expression of the ZMM28 protein in the absence of the intended trait, is not sufficient to 'assess the relevance of the field trials' for risk assessment.

In result the data provided in the case of these applications are inconclusive.

To fulfill the requirements of the EU GMO regulation, EFSA should have requested additional data on

• all biological active molecules that may emerge from new open reading frames;

• field trials that are up to-date with current agricultural practise and climatic conditions;

• targeted experiments to assess the effects of a broader range of environmental stressors;

• targeted experiments to assess the effects of higher-dosage spraying with the complementary herbicides;

• application of 'Omics' to investigate changes in the gene activity of the transgenes, as well as changes in the plants' own genes

• field trials in which the desired trait of higher yielding is exhibited.

Furthermore, in awareness of the absence of independent data on the GE maize, the production of the data should have been conducted under control of independent experts.

In conclusion, the data provided on the GE maize do not sufficiently represent the products intended for import. The data provided by the applicant and accepted by EFSA are insufficient to conclude on the impact of environmental conditions, stressors, herbicide applications and genetic backgrounds on gene expression and plant metabolism.

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

The current practice of EFSA fails to fulfill the legally requested standards as requested by EU Regulation 1829/2003. According to the Regulation, it is requested that food or feed derived from genetically modified organisms ("GMOs") must not have adverse effects on human health, animal health or the environment (Article 4(1)(a) and 16(1)(a) of the Regulation 1829/2003). As requested in Recital 10 of the Regulation "Thus, genetically modified food and feed should only be authorised for placing on the Community market after a scientific evaluation of the highest possible standard, to be undertaken under the responsibility of the European Food Safety Authority (Authority), of any risks which they present for human and animal health and, as the case may be, for the environment."

In the present application, the data provided by the applicant underline the need for further investigations. Data from only one year and 12 field trial sites are insufficient to represent the full range of conditions under which these plants will be grown. Since climate change is ongoing in regions where the plants are cultivated, the data from the applicant should allow to extrapolate for a period of 10 years (the duration of the intended market approval). Therefore, the data provided are invalid and do not allow to conclude the risk assessment.

More than half of the data from the compositional analysis (treated as well as untreated) assessed showed significant differences to the plants used as comparator. Further, phenotypic and agronomic analysis showed significant differences in five of ten (untreated) and four of 10 (treated) parameters. Thus, it is a valid hypothesis that the insertion of the gene constructs causes unintended effects on plant metabolism.

In regard to the intended enhanced yield potential provided by DP202216, field trials showed no higher yields for maize DP202216 × NK603 × DAS-40278-9. An absence of the yield gain was already shown in the data for the parental plant (EFSA, 2024). Only observing an increased expression of the ZMM28 protein in the absence of the intended trait, is not sufficient to 'assess the relevance of the field trials' for risk assessment.

It is likely that the plants if they would exhibit the desired trait will also show a different pattern of gene expression and a change in composition. At very least, their phenotypic characteristics will be significantly altered. Therefore, this problem, i.e. non-reproducibility of the intended trait, is particularly significant in molecular risk assessment as well as in compositional and phenotypic analysis.

To fulfill the requirements of the EU GMO regulation, EFSA should have requested additional data on

• field trials that are up-to-date with current agricultural practise and climatic conditions;

• the field trials conducted in all relevant regions where the GE cotton will be cultivated;

• targeted experiments to assess the effects of a broader range of environmental stressors;

• targeted experiments to assess the effects of higher-dosage spraying with the complementary herbicides;

• application of ‘Omics’ techniques to investigate changes in the plant composition.

Furthermore, in awareness of the absence of independent data on the GE maize, the production of the data should have been conducted under control of independent experts.

In conclusion, the data provided on the GE maize do not sufficiently represent the products intended for import. The data provided by the applicant and accepted by EFSA are insufficient to conclude on the impact of environmental conditions, stressors, herbicide applications and genetic backgrounds on plant composition and agronomic and phenotypic characteristics.

4. Toxicity

The current practice of EFSA fails to fulfill the legally requested standards as requested by EU Regulation 1829/2003. According to the Regulation, it is requested that food or feed derived from genetically modified organisms (“GMOs”) must not have adverse effects on human health, animal health or the environment (Article 4(1)(a) and 16(1)(a) of the Regulation 1829/2003). As requested in Recital 10 of the Regulation: “Thus, genetically modified food and feed should only be authorised for placing on the Community market after a scientific evaluation of the highest possible standard, to be undertaken under the responsibility of the European Food Safety Authority (Authority), of any risks which they present for human and animal health and, as the case may be, for the environment.”

Both, pesticide regulation and GMO regulation require a high level of protection for health and the environment. However, in practice, there are huge gaps in the risk assessment of herbicide resistant transgenic plants.

Since the application of the complementary herbicide is a regular part of agricultural practice in the cultivation of herbicide resistant plants, it can be expected that residues from spraying are always present in the harvest. Thus, in regard to herbicide resistant plants, specific assessment of residues from spraying with complementary herbicides has to be considered to be a prerequisite before any authorisation for genetically engineered plants can be granted. This concerns the actual toxicity of the specific residues as well as the effects of chronic exposure:

• The toxic profile of the residues from the application of the complementary herbicides in products derived from the transgenic crops can be largely different if compared to residues in other diets. This is especially relevant for those GE plants that exhibit resistance to several herbicides. Products derived from such plants will show a unique combination of active ingredients, their metabolites and their additives. Despite chronic exposure to this combination of residues has to be expected within the food chain, their chronic mixed toxicity was never assessed under pesticide regulation nor under GMO regulation. However, it is known that for several of the mixtures additive or synergistic toxicity has to be expected.

• The specific exposure to the residues from spraying with complementary caused by the consumption of transgenic plants can be largely different compared to other diets: it may cause a steady exposure to specific active ingredients in combination with metabolites and additives. Permanent exposure to this combination may have a specific impact on the gut microbiome, the immune system or other health issues related to specific organs. However, such effects were never investigated under pesticide nor under GMO regulation. However it

is known that for example herbicides containing glyphosate can have major impact on the gut microbiome. This finding is highly relevant for risk assessment of the present application: In 2019 in a study commissioned by EFSA, Parenti et al. (2019) state that “one of the most important drivers of immune response is the gut microbiota and other microbial constituent of the human body which are able to regulate host-pathogen balance and to produce systemic pro-inflammatory stimuli. The lifelong antigenic load represented by foods and bacteria/bacterial products leads to a profound remodeling of the gut microbiota and these changes are emerging as a driving force of the functional homeostasis of the immune system. As a matter of fact, a perturbation of the gut microbiota homeostasis due to irregular lifestyles, stress and age may lead to gut microbiota dysbiosis. This condition may predispose the host to metabolic disorders and inflammation.”

It follows that EFSA should have requested data on mixed toxicity and chronic exposure to the residues of spraying with the complementary herbicides.

More generally, EFSA never established any method to reliable assess mixed toxicity caused by a combination of active ingredients, their metabolites and their additives if sprayed as complementary herbicides. EFSA also never tried to assess health effects caused chronic exposure to this residues from spraying. This pertained scientific incompetence is not only a sign of maladministration (supported by the EU Commission), but may be understood as a matter of negligent endangerment.

In conclusion, EFSA practice for risk assessment of herbicide resistant transgenic plants needs to be revised completely. The risk assessment of the toxicity of present application is unacceptable.

5. Environmental risk assessment

Although the present application does not request approval for cultivation, environmental risk assessment is necessary and requested by EU Regulation 1829/2003 by referring to the standards of EU Directive 2001/18. Relevant issues are potential spread after spillage and environmental exposure via animal faeces or leftovers from food and feed processing.

The appearance of teosinte in Spain and France has to be considered in more detail. Maize volunteers can be found in the EU on a regular basis, and where maize and EU teosinte plants co-occur and flower synchronously, maize alleles (transgenic or not), can move into teosinte populations.

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 also interference from the genetic background that cannot be predicted from the assessment of the original event. This issue is relevant for gene flow from maize to teosinte, and from teosinte to maize.

Apart from that, the yield enhancement trait provided by DP202216 needs further assessment, as the zmm28 gene is not only associated with increased yield, but additionally with increased plant growth, increased photosynthetic capacity and improved nitrogen utilization. Therefore, the plants may show enhanced persistence in the environment.

It cannot be ruled out that plants growing from the kernels (intentionally or unintentionally), may result in the emergence of superweeds, thus posing serious risks to food security in Europe. Directive 2001/18 requires the applicant to outline measures preventing the misuse of genetically engineered plants. Therefore, the applicant should explain how they intend to prevent viable DP202216 × NK603 × DAS-40278-9 kernels (if imported into the EU) from being used in growing crops, and thus prevent gene flow to teosinte, as has been observed in Spain and France. While such misuse has to be considered to be illegal, the biological barriers that need to be overcome to produce these hybrids seem to be very low. The outcome would be plants that are not transient, such as maize volunteers, but which can persist and spread in the environment.

Without these data, the environmental risk assessment remains inconclusive (see also EFSA, 2025b).

6. Others

Monitoring

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

The method(s) shall be specific to the transformation event (hereafter referred to as 'event-specific') and thus shall only be functional with the genetically modified organism or genetically modified based product considered and shall not be functional if applied to other transformation events already authorised; otherwise the method cannot be applied for unequivocal detection/identification/quantification. This shall be demonstrated with a selection of non-target transgenic authorised transformation events and conventional counterparts. This testing shall include closely related transformation events.

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

Conflicts of interest

Testbiotech published a report on the current EFSA GMO Panel (Testbiotech, 2024). According to the investigation, members of the GMO panel with interests in developing genetically engineered plants have gained considerable influence within the authority. Testbiotech is therefore demanding corrections in the composition of the GMO Panel. Unless

balance is restored, any assessment by the Panel in its current composition has to be regarded as biased.

References

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