

Maize DP51291

Organisation: Testbiotech e.V. - Institute for Independent Impact Assessment of Biotechnology

Country: Germany

Type: Non Profit Organisation

Comments:

Introduction

The GMO Panel assessed maize DP51291 (EFSA, 2024a). The maize produces

- IPD072Aa toxin from the soil bacterium *Pseudomonas chlororaphis* (to act against corn rootworm),
- the phosphinothricin acetyl transferase (PAT) protein for tolerance to glufosinate-ammonium-containing herbicides,
- the PMI protein (as a selectable marker).

Consequently, the maize is resistant to glufosinate herbicides and toxic to corn rootworm. The genetic intervention involved a multistep process to introduce a ‘landing pad’ at the target site, where the gene constructs for the production of new proteins (new traits) are subsequently inserted.

1. Systematic literature review

A systematic review as requested in Regulation (EU) No 503/2013 was not provided by the applicant. The scoping review that was submitted does not appear to include a peer-reviewed publication with a clear explanation of the mode of action of the newly expressed insecticidal IPD072Aa toxin. Further, it has to be stated that there is a clear lack of independent science, i.e. all available scientific papers regarding event DP51291 and IPD072Aa toxin were written by the applicant.

2. Molecular characterisation

Maize DP51291 was developed using a multi-step method. In the first step, a specific genomic integration site (landing pad) was created with *Agrobacterium tumefaciens*. In step 2, five plasmids were introduced via microprojectile co-bombardment. In a third step, *A. tumefaciens* mediated transformation was used to insert the intended expression cassettes into the landing pad in the maize genome.

According to bioinformatic analysis, 113 bp were deleted from the genomic DNA of the maize during the process.

The genetic engineering process resulted in the emergence of several new open reading frames in the genome of the maize, five of which exceeded the allergenicity assessment threshold of 35% identity to known allergens. 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, EFSA states that proteins that may emerge from these DNA sequences would not raise safety concerns. However, the large number of ORFs indicates that there are signs of genomic perturbations caused by the multi-step process of genetic engineering - this should have prompted a higher level of scrutiny.

Thus, uncertainties remain about further biologically active substances resulting from the method of genetic engineering and the newly introduced gene constructs.

Gene expression under stress conditions

Environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). Expression of the additional enzymes was, nevertheless, only measured under field conditions at six locations in the US and Canada for one year (2021). It is evident that these weather conditions are not representative of all growing conditions, or of changing temperatures due to ongoing climate change. Therefore, new data on gene expression need to be made available. The data should also cover a much broader range of defined biotic and abiotic stressors to demonstrate stability in gene expression under sufficiently realistic conditions.

Consequently, the GE maize plants tested in field trials do not sufficiently represent the products intended for import. The data presented by the applicant are insufficient to conclude on the impact that environmental factors may have on gene expression, as laid down in EU Regulation 503/2013.

Impact of genetic backgrounds on gene expression

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

EFSA did not consider any of these issues. Consequently, the GE maize plants tested in the field trials do not sufficiently represent the products intended for import. As such, the data presented by the applicant are insufficient to conclude on the impact the genetic backgrounds may have on gene expression, as laid down in EU Regulation 503/2013.

Summary of molecular analysis

EFSA should have requested that the applicant use suitable methods to detect unintended genetic changes, and to assess all the biologically active molecules occurring at novel open reading frames. Data collection on gene expression should include the highest dosage of the

complementary herbicides that may be used in the countries of cultivation. Transgenic plants with differing genetic backgrounds should be grown in the field trials, and a broad range of defined environmental conditions should be applied. The plant material derived from such trials should be assessed with ‘Omics’ techniques to investigate changes in the gene activity of the transgenes, as well as changes in the plants’ own genes.

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

Implementing Regulation 503/2013 requests:

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

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

The data presented by Corteva do not meet the requirements of Implementing Regulation 503/2013: (1) the field trials were not conducted in all relevant regions where the GE maize will be cultivated, and no defined extreme weather conditions were taken into account; (2) the field trials did not take all relevant agricultural management practices into account; (3) not all relevant genetic backgrounds were taken into account.

Data on environmental factors and stress conditions - and their impact on plant composition and phenotype

Field trials to assess plant composition as well as agronomic and phenotypic characteristics of the GE maize were conducted for one year (2018) in the US and Canada, at eight (compositional analysis) resp. ten (agronomic performance) sites. Extreme weather conditions were reported from some of the fields, but no targeted investigation was carried out to, for example, investigate the impact of climate change. In order to assess possible compositional or agronomic changes, the plants should have been grown in various environmental conditions and exposed to well-defined environmental stress conditions, including taking other maize growing regions, e.g. Brazil, into account.

In light of the information available, we assume that the data provided do not sufficiently represent the agricultural practices and bio-regional conditions under which these plants are likely to be grown. For example, the plants also should have been grown in different environmental conditions in countries such as Brazil.

No experiments were requested to show to which extent specific environmental conditions influence plant composition and agronomic characteristics. Hence, the data made available do not allow conclusions (as requested in Implementing regulation 503/2013) on whether the

expected environmental conditions under which the plants are likely to be cultivated will influence the expression of the studied endpoints.

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

The complementary herbicide (glufosinate) was only applied once during the field trials. The dosage was chosen in accordance with the label recommendations (EFSA, 2024a). However, as Myiazaki et al. (2019) show, the herbicide applications are likely to differ across regions and in response to pressure by herbicide resistant plants.

Therefore, in the light of the information available, we assume that the data provided do not sufficiently represent the agricultural practices, e.g. higher dosages and repeated spraying.

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

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

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

EFSA did not take these issues into consideration. Consequently, the GE maize plants tested in field trials do not sufficiently represent the products intended for import. The data presented by the applicant are, therefore, insufficient to conclude on the impact that the genetic backgrounds may have on gene expression, as laid down in EU Regulation 503/2013.

Agronomic and phenotypic characteristics

Of the nine endpoints, the agronomic assessment resulted in one significant difference for maize DP51291 not treated with the intended herbicide, and one significant difference for maize DP51291 treated with the intended herbicide, as compared to the control maize line.

Data from compositional analysis

Maize DP51291 forage and grains harvested from the field trials were analysed for 69 different constituents (10 in forage and 59 in grains).

- For maize DP51291 not treated with the intended herbicide, statistically significant differences were identified in comparison to the conventional counterpart for 13 constituents in grain. The equivalence test between maize DP51291 and the non-GM conventional counterpart indicated that the differences in three constituents fell under equivalence Category III or IV.

- For maize DP51291 treated with the intended herbicide, statistically significant differences in comparison to the conventional counterpart were identified for 19 constituents (four in forage and 15 in grain). All these constituents fell under equivalence Category I or II except for phosphorus in forage, linoleic acid (C18:2) and manganese in grain which fell under equivalence Category III. Oleic acid (C18:1) in grain fell under equivalence Category IV.

In summary, statistical differences were found in almost one third of all constituents in maize DP51291 treated with the recommended dose of glufosinate (grain). Several constituents even fell under equivalence Category III or IV. In regard to the amino acid profile, equivalence could not be demonstrated for a key metabolic pathway. Similar changes in amino acid profile have been observed for maize event DP23211 (which also produces IPD072Aa and PAT), thus the hypothesis that the genes of interest have unintended effects on plant metabolism seems plausible. These findings should have prompted further investigations in regard to the equivalence of maize DP51291 with its isogenic parent.

Given the above reasoning on the impact of environmental factors, herbicide application and genetic backgrounds as well as the large number of significant findings, EFSA should have requested more data: data on agronomic and phenotypic endpoints should be generated from a wider range of clearly defined stress factors, including all relevant agricultural practices and genetic backgrounds.

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

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

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

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

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

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

4. Toxicity

Implementing Regulation 503/2013 requests:

“Toxicological assessment shall be performed in order to:

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

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

“In accordance with the requirements of Articles 4 and 16 of Regulation (EC) No 1829/2003, the applicant shall ensure that the final risk characterisation clearly demonstrates that:

(a) the genetically modified food and feed has no adverse effects on human and animal health;”

IPD072Aa protein

As far as the IPD072Aa protein is concerned, there are some important differences, but also some similarities, in comparison to Bt toxins currently produced by transgenic plants. These need to be taken into account in risk assessment.

1. The source

Introducing these proteins into agriculture and the food chain cannot fully rely on previously gained experience. The source organism of the gene coding for the IPD072Aa protein (*Pseudomonas chlororaphis*) is used in agriculture to protect plants by producing compounds that inhibit fungal growth insects and nematodes. EFSA assessed a strain of *P. chlororaphis* in 2017 and identified several data gaps regarding mammalian toxicity and ecotoxicology, amongst others (EFSA, 2017).

2. Mode of action

While the mode of action of Bt toxins, before introduced into transgenic plants, was already a matter of investigations and explored to some detail, this is not the case with the toxin from *P. chlororaphis*. So far, the mode of action of this insecticide is only partly understood. A recent paper by Corteva scientists on this subject gives some details, but still has to admit that:

“Attempts to observe pore formation by IPD072Aa in artificial lipid membranes failed to reveal pore forming activity (Nelson, unpublished observations). [...] Similarly, attempts to identify the plasma membrane receptor for IPD072Aa using co-precipitation and yeast two-hybrid approaches failed to identify a protein interactor that serves as a valid functional receptor (data not shown). [...] The molecular mechanism of IPD072Aa toxicity to WCR larvae, including the identity of the membrane receptor to which it binds will continue to be evaluated” (Jiménez-Juárez et al., 2023).

In addition, all data available seem to stem from experts working for the applicant. The lack of insight into the mode of action of IPD072Aa toxin is also underlined by comments from the experts from the Member States (EFSA, 2024b).

3. Combinatorial or synergistic factors impacting toxicity and allergenicity

It is known from toxicity of Bt toxins that plants constituents such as protein inhibitors or other cofactors substances can largely enhance its toxicity (MacIntosh et al., 1990; Pardo-López et al., 2009). Therefore, to determine ‘no observed effect concentration’ or ‘no observed effect dose’, it is not sufficient to use the proteins in isolation as produced by bacteria. Instead, it is necessary to take into account the real conditions of exposure, such as in combination with plant protein inhibitors. The residues from spraying with glufosinate should also be considered. This is obligatory for determining chronic and subchronic toxicity, immunogenicity (allergenicity), impact on microorganisms (intestinal microbiome or soil organisms) as well as the effects on non-target organisms.

b) Effects of residues from spraying with complementary herbicide specific to GE plants

Glufosinate is classified for presumed reproductive toxicity. There are also indications of additive and synergistic effects caused by residues from spraying (<https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances/details/79>). The marketing of glufosinate in the EU was terminated in 2019. Currently, the EU has a maximum residue level (MRL) of 0.1 mg/kg for glufosinate in maize imported from third countries. Therefore, the notifier should have requested proof that the MRLs established in the EU for residue levels of the herbicide in maize imported from third countries are not exceeded.

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

EU legal provisions, such as Regulation 1829/2003 (and Implementing Regulation 503/2013), state that “any risks which they present for human and animal health and, as the case may be, for the environment” have to be avoided. Therefore, potential adverse effects resulting from combinatorial exposure of various potential stressors need to be tested.

In regard to food and feed safety, EFSA (2020) considers microbiomes to be highly relevant to the health status of their hosts. Therefore, it is desirable to understand the importance of their role in risk assessment. EFSA expects that gut microbiome research (not only in the case of GE plants) will play a relevant role in regulatory science with potential implications for future risk assessments and predictive risk models. As EFSA states: “considering that the gut microbiome is a biological component directly and indirectly involved in the metabolism of food/feed components and chemicals and in the protection of the host against adverse environmental exposure, it would be useful to establish criteria on how to evaluate the potential adverse impacts of perturbators on this defensive barrier, and consequently, on human/animal health.”

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

Dong et al. (2020) showed that glufosinate can have a severe impact on the microbiome. However, no attempts have been made to integrate the microbiome into the risk assessment of food and feed derived from the GE maize. This is in direct contradiction to Regulation 1829/2003 which requests “genetically modified food and feed should only be authorised for placing on the Community market after a scientific evaluation of the highest possible standard, to be undertaken under the responsibility of the European Food Safety Authority (Authority), of any risks which they present for human and animal health and, as the case may be, for the environment.” (Recital 9).

EU legal provisions such as Regulation 1829/2003 (as well as Implementing Regulation 503/2013) state that “any risks which they present for human and animal health and, as the case may be, for the environment” have to be avoided.

In addition, cumulative effects (mixtures of GE plants in one diet) may play a decisive role. For example, insecticidal toxins or residues from spraying with other herbicides may contribute to synergistic effects that can be decisive for the overall toxicity of a given diet.

Furthermore, findings by Christ et al. (2017) showing that the PAT/BAR enzyme may also acetylate endogenous amino acids, should have been the starting point for further investigations.

5. Environmental risk assessment

a) IPD072Aa toxin

The specificity of the IPD072Aa toxin was tested on several non-target insects and some grain-feeding mammals (Boeckmann et al., 2021). However, the test material itself was produced in bacteria in isolated form. Therefore, this approach leaves aside the real routes of exposure via plant material, which will always include protein inhibitors that may strongly increase toxicity. In addition, other potential co-factors and stressors typically present in the receiving environments were ignored. No data were made available on whether the protein can accumulate in the food webs, or persist and accumulate in the environment (e. g. the soil).

In peer-reviewed papers published by the applicant, there were several significant findings in regard to non-target organisms (Boeckmann et al., 2019, 2021). Negative effects were found for example in springtails and species of ladybird beetles. These results should be the starting point for risk assessment by non-corporate scientists.

Available data shows that IPD072Aa is stable and biologically active after heat treatment up to 95°C (Carlson et al. 2019). Therefore, environmental exposure via waste material from processing must be anticipated. Gastric fluids in mammals are likely to degrade the toxin but studies with a qualitative proof (i.e. bioassays) are missing. In addition, the information on non-target soil organisms, which would be main group affected by waste and manure containing IPD072Aa, are lacking. Data should be made available regarding:

- the concentration of IPD072Aa in the manure of livestock fed with DP51291,
- the distribution of IPD072Aa in the environment via wastewater and manure into soil and waterbodies,
- the effect of IPD072Aa on non-target organisms, especially soil and water organisms, including additive and synergistic effects on lethal and sub-lethal fitness parameters.

b) Teosinte

The appearance of teosinte in Spain and France (see Testbiotech, 2016; Trtikova et al., 2017) has to be considered in more detail. Maize volunteers can be found in the EU on a regular basis, as reported by Palau-del-màs et al. (2009) in Spain or Pascher (2016) in Austria.

Testbiotech is aware of an EFSA (2022) opinion regarding the teosinte situation in France and Spain. Here, EFSA comes to the conclusion:

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

This opinion not sufficiently backed by science: 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 (Bauer-Pankus et al., 2020). This issue is relevant for gene flow from maize to teosinte, and from teosinte to maize.

In addition, new evidence has become available since EFSA prepared its opinion (Arias-Martin et al., 2019, 2022, 2024). This new evidence was also assessed by EFSA (2024c). The new EFSA assessment shows that the environmental risk assessment of MON95275 needs to be revised. EFSA (2024c) states that “the evidence indicates that the hybridisation potential between maize MON 810 and teosinte found in Spain can be greater than assumed previously”, that the hybrid offspring “may be more vigorous (e.g. taller, thicker stem, more leaves) than teosinte” and “that teosinte/maize hybrids express the Cry1Ab protein at similar

levels than maize MON 810.” EFSA (2024c) has further requested data to assess the invasiveness and hybridisation potential of maize MON 810 and teosinte in field conditions.

These new data not only apply to MON810 and Cry1Ab toxin, but also to other maize events. Until further data on this subject is available, the EFSA conclusion stated in the DP51291 assessment that “the GMO Panel is of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties” (EFSA, 2024a), does not reflect the new scientific evidence. The opinion should therefore be retracted.

Testbiotech warns that viable kernels from the transgenic maize should not be imported if this data is not available. It cannot be ruled out that plants growing from the kernels (intentionally or unintentionally), may very easily 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 DP51291 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.

Whatever the case, without detailed consideration of the hazards associated with potential gene flow from maize to teosinte, and from teosinte to maize, no conclusion can be drawn on the environmental risks of maize DP51291.

Consequently, the EFSA environmental risk assessment is not acceptable.

6. Others

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

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

If approval for import is given, the applicant has to ensure that post-market monitoring (PMM) is developed to collect reliable information on the detection of indications showing whether any (adverse) effects on health may be related to GM food or feed consumption. Thus, the monitoring report should at very least contain detailed information on: i) actual volumes of the GE products imported into the EU, ii) the ports and silos where shipments of the GE products were unloaded, iii) the processing plants where the GE products was transferred to, iv) the amount of the GE products used on farms for feed, and v) transport routes of the GE products. Environmental monitoring should be run in regions where viable material of the GE products such as kernels are transported, stored, packaged, processed or

used for food/feed. In case of losses and spread of viable material (such as kernels) all receiving environments need to be monitored. Furthermore, environmental exposure through organic waste material, by-products, sewage or faeces containing GE products during or after the production process, and during or after human or animal consumption should be part of the monitoring procedure.

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

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

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

Conflicts of interest

Testbiotech recently published a report on the current EFSA GMO Panel (Testbiotech, 2024). According to our investigation, members of the GMO panel with interests in developing genetically engineered plants have recently gained considerable influence. 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

Arias-Martín M., Escorial Bonet M.C., Loureiro Beldarraín I. (2024) Teosinte introducido en España y maíz Bt: tasa de hibridación, fenología y cuantificación de toxina Cry1ab en los híbridos. *Rev Ciênc Agrá* 47(1): 297–301. <https://doi.org/10.19084/rca.35040>

Arias-Martín M., Escorial M.C., Chueca M. C., Loureiro I. (2019) Cruzabilidad entre maíz (*Zea mays*) y teosinte (*Zea mays* ssp. *mexicana* y *Zea mays* ssp. *parviglumis*) y caracterización morfológica de los híbridos obtenidos. XVII Congreso de la Sociedad Española de Malherbología: libro de actas, Vigo, 8-10 de octubre de 2019. Pedrol Bonjoch N. (ed.), González Puig C. (ed.). ISBN 978-84-120734-3-0, pp. 396-401. <https://dialnet.unirioja.es/servlet/articulo?codigo=7233037>

Arias-Martín M., Escorial M. C., Loureiro I. (2022) Hybridization between maize and the teosinte species *Zea mays* ssp. *mexicana* and *Zea mays* ssp. *parviglumis*. *Actas XVIII Congreso de la Sociedad Española de Malherbología 2022*. Mérida, Spain, p. 141. https://cdn.website-editor.net/s/8e89c5488128400daf669d61abfda791/files/uploaded/libro-de-resumenes_congreso-seea_2022.pdf

Bauer-Panskus A., Miyazaki J., Kawall K., Then C. (2020) Risk assessment of genetically engineered plants that can persist and propagate in the environment. *Environ Sci Europ*, 32(1): 1-15. <https://doi.org/10.1186/s12302-020-00301-0>

Benevenuto R.F., Venter H.J., Zanatta C.B., Nodari R.O., Agapito-Tenfen S.Z. (2022) Alterations in genetically modified crops assessed by omics studies: Systematic review and meta-analysis. *Trends Food Sci Technol*, 120: 325-337. <https://doi.org/10.1016/j.tifs.2022.01.002>

Boeckman C. J., Huang E., Sturtz K., Walker C., Woods R., Zhang J. (2019) Characterization of the spectrum of insecticidal activity for IPD072Aa: a protein derived from *Pseudomonas chlororaphis* with activity against *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). *J Econ Entomol*, 112(3): 1190-1196. <https://doi.org/10.1093/jee/toz029>

Boeckman C.J., Anderson J.A., Linderblood C., Olson T., Roper J., Sturtz K., ... & Woods R. (2021) Environmental risk assessment of the DvSSJ1 dsRNA and the IPD072Aa protein to non-target organisms. *GM Crops & Food*, 12(1): 459-478. <https://doi.org/10.1080/21645698.2021.1982348>

Carlson A.B., Mathesius C.A., Ballou S., Boeckman C.J., Gunderson T.A., Mirsky H.P., ... & Delaney B. (2019) Safety assessment of coleopteran active IPD072Aa protein from *Pseudomonas chlororaphis*. *Food Chem Toxicol*, 129: 376-381. <https://doi.org/10.1016/j.fct.2019.04.055>

Dong T., Guan Q., Hu, W., Zhang, M., Zhang, Y., Chen, M., ... & Xia, Y. (2020) Prenatal exposure to glufosinate ammonium disturbs gut microbiome and induces behavioral abnormalities in mice. *J Hazard Mat*, 389: 122152. <https://doi.org/10.1016/j.jhazmat.2020.122152>

EFSA (2012) Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function. In: *EFSA J*, 10(10): 2943. <https://doi.org/10.2903/j.efsa.2012.2943>

EFSA (2017) Conclusion on the peer review of the pesticide risk assessment of the active substance *Pseudomonas chlororaphis* strain MA 342. *EFSA J*, 15(1): 4668. [doi:10.2903/j.efsa.2017.4668](https://doi.org/10.2903/j.efsa.2017.4668)

EFSA (2020) Editorial: Exploring the need to include microbiomes into EFSA's scientific assessments. *EFSA J*, 18(6): e18061. <https://doi.org/10.2903/j.efsa.2020.e18061>

EFSA (2022) Statement on the update of environmental risk assessment conclusions and risk management recommendations of EFSA (2016) on EU teosinte. *EFSA J*, 20(4): 7228. <https://doi.org/10.2903/j.efsa.2022.7228>

EFSA (2024a) Assessment of genetically modified maize DP51291 (application GMFF-2021-0071). *EFSA J*, 22(11): e9059. <https://doi.org/10.2903/j.efsa.2024.9059>

EFSA (2024b) Comments and opinions submitted by Member States during the three-month consultation period [Supporting information]. <https://doi.org/10.2903/j.efsa.2024.8483>

Gao H., Mutti J., Young J.K., Yang M., Schroder M., Lenderts B., ... & Chilcoat N.D. (2020) Complex trait loci in maize enabled by CRISPR-Cas9 mediated gene insertion. *Front Plant Sci*, 11: 535. <https://doi.org/10.3389/fpls.2020.00535>

Jiménez-Juárez N., Oral J., Nelson M.E., Lu A.L. (2023) IPD072Aa from *Pseudomonas chlororaphis* targets midgut epithelial cells in killing western corn rootworm (*Diabrotica virgifera virgifera*). *Appl Environ Microbiol*, 89(3): e01622-22. <https://doi.org/10.1128/aem.01622-22>

Lohn A.F., Trtikova M., Chapela I., Van den Berg J., du Plessis H., Hilbeck A. (2020) Transgene behavior in *Zea mays* L. crosses across different genetic backgrounds: Segregation patterns, cry1Ab transgene expression, insecticidal protein concentration and bioactivity against insect pests. *PLoS ONE*, 15(9): e0238523. <https://doi.org/10.1371/journal.pone.0238523>

MacIntosh, S.C., Kishore, G.M., Perlak, F.J., Marrone, P.G., Stone, T.B., Sims, S.R., Fuchs, R.L. (1990) Potentiation of *Bacillus thuringiensis* insecticidal activity by serine protease inhibitors. *J. Agric Food Chem*, 38: 1145-1152. <https://doi.org/10.1021/jf00094a051>

Miyazaki J., Bauer-Panskus A., Bøhn T., Reichenbecher W., Then C. (2019) Insufficient risk assessment of herbicide-tolerant genetically engineered soybeans intended for import into the EU. *Environ Sci Eur*, 31(1): 1-21. <https://doi.org/10.1186/s12302-019-0274-1>

Palau-del-màs M., Peñas G., Melé E., Serra J., Salvia J., Pla M., Nadal A., Messeguer J. (2009) Effect of volunteers on maize gene flow. *Transgenic Res*, 18(4): 583-594. <https://doi.org/10.1007/s11248-009-9250-7>

Pardo-López L., Muñoz-Garay C., Porta H., Rodríguez-Almazán C., Soberón M., Bravo A. (2009) Strategies to improve the insecticidal activity of Cry toxins from *Bacillus thuringiensis*. *Peptides*, 30(3): 589-595. <https://doi.org/10.1016/j.peptides.2008.07.027>

Parenti M.D., Santoro A., Del Rio A., Franceschi C. (2019) Literature review in support of adjuvanticity/immunogenicity assessment of proteins. *EFSA Supporting Publications*, 16(1): 1551E. <https://doi.org/10.2903/sp.efsa.2019.EN-1551>

Pascher K. (2016) Spread of volunteer and feral maize plants in Central Europe: recent data from Austria. *Environ Sci Eur*, 28(1): 28-30. <https://doi.org/10.1186/s12302-016-0098-1>

Testbiotech (2016) Cultivation of genetically engineered maize: Risks not under control - Overview: Why the EU should not allow the cultivation of transgenic maize engineered to produce insecticidal toxins. *Testbiotech Background*, <https://www.testbiotech.org/node/1759>

Testbiotech (2021) Risk assessment of GE plants in the EU: Taking a look at the 'dark side of the moon'. <https://www.testbiotech.org/content/risk-assessment-ge-plants-eu-taking-look-dark-side-moon>

Testbiotech (2024) NGT plant developers take over the EFSA GMO Panel, <https://www.testbiotech.org/publikation/ngt-plant-developers-take-over-the-efsa-gmo-panel/>

Trtikova, M., Wikmark, O. G., Zemp, N., Widmer, A., Hilbeck, A. (2015) Transgene expression and Bt protein content in transgenic Bt maize (MON810) under optimal and stressful environmental conditions. PLoS ONE 10(4): e0123011.
<https://doi.org/10.1371/journal.pone.0123011>

Trtikova M., Lohn A., Binimelis R., Chapela I., Oehen B., Zemp N., Widmer A., Hilbeck A. (2017) Teosinte in Europe – searching for the origin of a novel weed. Sci Rep, 7: 1560.
<https://doi.org/10.1038/s41598-017-01478-w>