

TESTBIOTECH Background 17 - 02 - 2024

Assessment of genetically engineered maize DP 915635 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2020-172) by Pioneer Hi-Bred International

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Introduction

The GMO Panel assessed the herbicide tolerant maize DP915635, which produces an insecticide and the selectable marker gene PMI (EFSA, 2024a). This event was developed to confer resistance to glufosinate and produce the IPD079Ea toxin derived from the *Ophioglossum pendulum* fern. The toxin is intended to be active against corn rootworm. The genetic intervention involved a multistep process using CRISPR/Cas 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

The applicant did not provide a systematic review as requested in Regulation (EU) No 503/2013. A number of selected studies were included. There appears to be no peer-reviewed study on the mode of action of the newly expressed insecticidal toxin. Nor was a publication provided on the risks of unintended effects associated with the specific genetic engineering processes used to produce DP915635.

2. Molecular characterisation

This was a two-step process:

1. Step (not related to the trait):

Microprojectile co-bombardment, which involved CRISPR-Cas9 and the gene construct for a ‘landing pad’ sequence, to be inserted in the maize genome by homology-directed repair (HDR). This first step included four plasmids: one plasmid for introducing the gene scissors (CRISPR/Cas), and one with sequences for the ‘landing pad’. The ‘landing pad’ utilizes the flippase (FLP) recombinase and FLP recognition targets (FRT) to insert a gene in the target site. The other two plasmids were introduced to produce proteins to improve regeneration (the WUS2 and the ODP2 protein). The DNA sequence for the ‘landing pad’ was supposed to be integrated permanently; the other three plasmids were meant to be expressed only transiently.

It is important to acknowledge that unless adequate gene sequencing methodology is available, specific unintended genetic changes and associated risks may remain undetected. In this context, it is not sufficient to simply assume that the types of unintended genetic changes may be no different to conventional breeding (EFSA, 2024b). It is essential in this respect to take the site of the genetic

change into account, and to consider whether there are, e. g. inversions, insertions or deletions that are otherwise either unlikely or less likely to occur (Koller & Cieslak, 2023).

2. Step (related to the trait):

The ‘landing pad’ and the flippase (FLP) recombinase in combination with the FLP recognition targets (FRT) were used for the insertion of the gene constructs needed to establish the desired traits. *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) transformation was used for the insertion of the expression cassettes into the ‘landing pad’ in the maize genome. Three gene cassettes are intended to express three new proteins:

- The phosphomannose isomerase (PMI) gene from *E. coli* to facilitate detection of the successfully engineered plants (marker gene);
- a maize-optimised version of the PAT coding sequence of the phosphinothricin acetyltransferase gene (mo-pat) from *Streptomyces viridochromogenes*;
- the coding sequence of the insecticidal protein gene ipd079Ea. The fern *Ophioglossum pendulum* is the donor plant for this protein. It is an epiphyte that grows mainly in wet tropical habitats. The DNA sequence is linked to promoters that are primarily active in the roots.

In regard to risk assessment, the flippase system is intended to allow targeted insertion at the ‘landing pad’. However, its usage can also be associated with “truncations, insertion of other plasmid fragments or rearrangement of the template DNA” (Gao et al., 2020). Unintended effects may also occur at off-target sites. Therefore, the application of suitable methods to detect off-target genetic changes are essential. All open reading frames should be carefully investigated for emergent unintended biologically active molecules.

Furthermore, as shown by Gao et al. (2020), the expression of the gene constructs (inserted into the ‘landing pad’) depends on the genetic background of the plants. “It is noteworthy that expression at identical sites was significantly different across different genetic backgrounds...”. Therefore, gene expression should be investigated, including a broad range of different genetic backgrounds and environmental conditions.

The material used to investigate gene expression was produced in 2019 at five locations in the USA, and at one location in Canada. There was no targeted investigation into the impact of different environmental conditions (abiotic stressors) or differing genetic backgrounds. This is not acceptable since the plants are intended for cultivation in countries, such as Brazil, where other transgenic varieties with differing comparative relative maturity (CRM) and different genetic backgrounds are cultivated.

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 the applicant 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 extreme weather conditions were taken into account; (2) the field trials did not take all relevant agricultural management practices into account; (3) different genetic backgrounds inheriting the transgenes were not taken into account.

Data on environmental factors and stress conditions

Field trials to assess plant composition as well as agronomic and phenotypic characteristics of the GE maize were conducted in the US and Canada for one year (2019); at eight (compositional analysis) or eleven (agronomic performance) sites. Some extreme weather conditions were reported in some fields, but no targeted investigation was carried out to, for example, investigate the impact of climate change conditions. In order to assess changes in gene expression, the plants should have been grown in various environmental conditions, and exposed to well-defined environmental stress conditions, including taking maize growing regions, such as Brazil, into account.

From 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 are also intended for cultivation in different environmental conditions in countries such as Brazil.

No experiments were requested to show the extent to which specific environmental conditions influence plant composition and agronomic characteristics. Hence, the data made available do not allow conclusions to be drawn (as requested in Implementing regulation 503/2013), or whether the expected environmental conditions in which the plants are likely to be cultivated will influence the expression of the studied endpoints.

Data on herbicide application rates

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 from herbicide resistant plants.

Therefore, from 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 biological characteristics of the plant, as requested in EU Regulation 503/2013.

Data from different genetic backgrounds and their impact on plant composition as well as agronomic and phenotypic characteristics

As shown by Gao et al. (2020), the expression of the gene constructs inserted into the ‘landing pad’ depends on the genetic background of the plants.

Therefore, gene expression in a broad range of different genetic backgrounds should be investigated. Maize varieties with differing genetic backgrounds (different maturity groups) were only grown in the control group, while the transgenic plants all had the same genetic background. This is not acceptable as the plants are intended for cultivation in countries, e. g. Brazil, where other varieties with differing comparative relative maturity (CRM) will be grown under different environmental conditions.

Agronomic and phenotypic characteristics

According to EFSA, statistical analysis was applied to only eight endpoints, with the following results:

- For maize DP915635 (not treated with the intended herbicide): statistically significant differences were detected for early stand count and days to flowering.
- For maize DP915635 (treated with the intended herbicide): no statistically significant differences with the comparator were detected.

We do not consider these data to be sufficient for risk assessment, as they were derived from transgenic plants with only one genetic background and one herbicide application, without taking a broad range of defined environmental stress conditions into account.

Data from compositional analysis

According to EFSA, statistical analysis was applied to a total of 70 constituents (10 in forage and 60 in grain).

- For maize DP915635 not treated with the intended herbicides: statistically significant differences with the non-GM comparator were found for eight endpoints (all in grains).
- For maize DP915635 treated with the intended herbicides: statistically significant differences with the non-GM comparator were found for 11 endpoints (1 in forage and 10 in grains). Only one of these differences (crude protein in forage) was considered in further assessment (so-called equivalence category III).

We do not consider these data to be sufficient for risk assessment, as they were derived from transgenic plants with only one genetic background and one herbicide application, without taking a broader range of defined environmental stress conditions into account.

Furthermore, the material derived from the plants should have been assessed using ‘Omics’ techniques to investigate changes in the gene activity of the transgene and the plant genome, including investigating changes in metabolic pathways and the emergence of unintended biologically active gene products (see Benevenuto et al., 2022).

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 draw conclusions on how environmental stressors, herbicide applications and genetic backgrounds will impact gene expression, plant metabolism, plant composition or agronomic and phenotypic characteristics.

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

Based on the available data, no final conclusions can be drawn on the safety of the plants. The data neither fulfill the requirements of Implementing Regulation 503/2013 nor Regulation 1829/2003. This is also underlined in several statements made by experts from Member States (EFSA, 2024b).

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;”*

a) Assessment of the toxin IPD079Ea

There are some important differences, but also some similarities in comparison to Bt toxins currently produced by transgenic plants, which need to be taken into account in risk assessment.

1. The source and the previous range of distribution

Bt toxins can to a certain extent be considered ubiquitous in soil bacteria. However, this is not the case with the *Ophioglossum pendulum* toxin. This fern is not a part of European flora and has never previously been introduced into the food chain. Therefore, we cannot draw on previous experience gained from introducing these proteins into agriculture and the food chain.

2. Existing experience

Bt toxins were already being used in insecticidal spray prior to being introduced into transgenic plants. This is not the case with *Ophioglossum pendulum* toxin. Introducing these proteins into agriculture and the food chain as insecticides would first of all require a full assessment of the proteins under pesticide regulation.

3. Mode of action

The mode of action of Bt toxins was already investigated and explored in detail prior to their introduction into transgenic plants. This is not the case with *Ophioglossum pendulum* toxin. As yet, the mode of action of this insecticide has only been poorly described (see for example Barry et al., 2023). In addition, all the available data seem to originate from experts working for the applicant. The introduction of these proteins into agriculture and the food chain would require a lot more data on the mode of action and specificity of the toxins. This is also underlined in comments made by experts from member states (EFSA, 2024b).

4. Combinatorial or synergistic factors impacting toxicity and allergenicity

It is known that plant constituents, such as protein inhibitors or other co-factors, can greatly enhance the toxicity of Bt toxins (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 produced by the bacteria in isolation. Instead, it is necessary to take the real conditions of exposure into account, e. g. in combination with plant protein inhibitors. In addition, the residues from spraying with glufosinate should also be considered. These findings are relevant for determining chronic and subchronic toxicity, immunogenicity (allergenicity), the impact on microorganisms (intestinal microbiome or soil organisms) and the effects on non-target organisms.

In conclusion, the data is insufficient to assess the specificity of the toxin, or the toxicity of the protein in isolation and its allergenic potential.

Furthermore, whole food and feed feeding studies should be carried out only after the mode of action, the specificity, the ‘no observed effect concentration’ or ‘no observed effect dose’ of the proteins have been explored.

Cumulative effects (mixtures of GE plants in one diet) should also be considered. For example, Bt toxins or residues from spraying may contribute to synergistic effects that can be decisive for the overall toxicity of a given diet.

All in all, the toxicity assessment carried out by EFSA is not conclusive and cannot be accepted. This is also underlined in several statements made by experts from member states (EFSA 20204b)

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

The residues from spraying were considered to be outside the remit of the GMO Panel. However, without taking the assessment of these residues into account, conclusions cannot be drawn on the safety of the imported products.

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.

Glufosinate has been shown to impact or disturb the microbiome (Dong et al., 2020). This can have substantial impact on the long-term toxicity (mixed toxicity) of whole food and feed derived from the maize. In general, the microbiome can be seen as a common network of life, encompassing and closely interacting with plants, animals and humans. Microbial networks are thought to have co-evolved with their hosts and have developed a mutualistic relationship that benefits both the host and microorganisms. They act at the interphase and communicate between the organisms and their wider environment while at the same time being part of an organism's closer environment.

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.”*

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, Bt 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.

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

5. Environmental risk assessment

The specificity of the toxin was tested on several non-target insects and some mammals that eat grain (O'Neill et al., 2024). However, the test material was produced by E.coli in isolated form. Therefore, this approach ignores 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 that are typically present in the receiving environments were also ignored. No data were made available to show whether the protein can accumulate in food webs, or persist and accumulate in the environment (e. g. the soil). This means there is a lack of basic information needed for environmental risk assessment.

Furthermore, 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 Palaudelmàs 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 need 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 the genetic background that cannot be predicted from the assessment of the original event (Bauer-Panskus et al., 2020). Furthermore, as mentioned, gene expression at the ‘landing pad’ can depend on the genetic background of the transgenic plants (Gao et al., 2020). This issue is relevant for gene flow from maize to teosinte, and 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 cannot be regarded as 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, 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 any indications of whether any (adverse) effects on health could 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; 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, this 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 on whether the provisions of GMO regulation (esp. 503/2013) are fulfilled. We are making this comment after our recent experience 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 an 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 simply ‘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.

References

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>

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 (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 DP915635 for food and feed uses, under regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2020-172). *EFSA J*, 22(1): e8490. <https://doi.org/10.2903/j.efsa.2024.8490>
- EFSA (2024b) Comments and opinions submitted by Member States during the three-month consultation period [Supporting information]. <https://doi.org/10.2903/j.efsa.2024.8490>
- 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>
- Koller F., & Cieslak M. (2023) A perspective from the EU: unintended genetic changes in plants caused by NGT - their relevance for a comprehensive molecular characterisation and risk assessment. *Front Bioeng Biotechnol*, 11: 1276226. <https://doi.org/10.3389/fbioe.2023.1276226>
- 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>
- O'Neill B.F., Boeckman C., LeRoy K., Linderblood C., Olson T., Woods R., Challender M. (2024) An environmental risk assessment of IPD079Ea: a protein derived from *Ophioglossum pendulum* with activity against *Diabrotica* spp. in maize. *GM Crops & Food*, 15(1): 15-31. <https://doi.org/10.1080/21645698.2023.2299503>
- 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 adjuvant/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 (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>

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>