

TESTBIOTECH Background 15 - 12 - 2022

Assessment of genetically modified maize MON 95379 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2020-170) by Bayer

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Introduction

The GMO Panel assessed maize MON 95379 (EFSA, 2022a). The maize contains genes expressing two new insecticidal Bt proteins, Cry1B.868 and Cry1Da₇.

Cry1Da₇ and Cry1B.868 are modified versions of Bt toxins which do not occur in nature.

Cry1Da₇ shows enhanced activity against corn earworm, Cry1B.868 was changed to make the Bt toxin more effective against fall armyworm.

1. Systematic literature review

A systematic review as referred to in Regulation (EU) No 503/2013 was not provided by the applicant. Based on preliminary information, the GMO Panel agreed that there was only limited value in undertaking a systematic review (EFSA, 2022a). This is not acceptable. The applicant should have conducted a review including data on each of the toxins produced in the plants. Further, the EFSA statement that “*literature searches did not identify any relevant peer-reviewed publications on maize MON 95379*” can hardly be taken seriously. A simple literature search instantly delivers several scientific papers on both the event and the newly developed Bt toxins. Moreover, some of the scientific papers were also mentioned by experts during the consultation with Member States and even by EFSA itself (EFSA, 2022b).

2. Molecular characterisation

Maize MON 95379 produces two new Bt toxins with no history of safe use. Cry1B.868 gene is made up of domains I and II of Cry1Be of *Bacillus thuringiensis*, of domain III of Cry1Ca of *Bacillus thuringiensis* subsp. *aizawai* and the C-terminal protoxin region of the Cry1Ab protein of *Bacillus thuringiensis* subsp. *Kurstaki*. The Cry1Da₇ gene is a mutated sequence with regard to four amino acids of the Cry1Da protein of *Bacillus thuringiensis* subsp. *aizawai*. The maize was produced in a two-step process. The two Bt genes were transferred into two separate lines which were then crossed. A marker gene (providing glyphosate resistance) was excised using a cre/lox system. The cre recombinase was then segregated away through conventional breeding.

Bioinformatics analyses (next generation sequencing) revealed two open reading frames (ORFs) with allergenic potential. However, EFSA considers the allergenicity risk to be low, as they do not contain a start codon.

Gene expression data show a very wide range of Bt content in forage and grain, even though no extreme weather conditions were reported. The high variability of Bt content should have led to further investigations.

Environmental stress can cause unexpected patterns of expression in the newly introduced DNA (see, for example, Trtikova et al., 2015). However, the expression of the additional enzymes was only measured under field conditions in the US for one year. It is unclear, to which extent specific environmental conditions will influence the overall concentration of the enzymes in the plants. 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.

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, it appears that the data on gene expression were confined to a single variety. Therefore, EFSA should also have requested additional data from transgenic maize varieties, e.g. those cultivated in South America.

However, EFSA has not taken 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 of the genetic backgrounds on gene expression as requested in EU Regulation 503/2013.

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

Implementing Regulation 503/2013 requests:

“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 Bayer 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 not all relevant extreme weather conditions were taken into account (such as drought); (2) not all relevant genetic backgrounds were taken into account.

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

Phenotypic data was obtained from GM maize treated with pesticides. However, the use of agrochemicals may impact fitness and reduce stress such as competition or herbivory. The current test design is, therefore, not appropriate for comparative investigations of GMO fitness in non-agricultural environments.

Field trials to assess plant composition as well as agronomic and phenotypic characteristics of the

GE maize were only conducted in the US for one year. No extreme weather conditions were reported from the field trials, so no conclusions to be drawn on how gene expression will be affected by more severe climate stress due to drought, watering or high temperatures. In order to assess changes gene expression, the plants should have been grown in various environmental conditions and exposed to well-defined environmental stress conditions.

It should not be overlooked that, for example, Brazil is among the most important countries for maize imports into the EU: Brazil is a major producer of genetically engineered maize and is one of the largest exporters of maize to the EU (Commission Committee for the Common Organisation of Agricultural Markets, 2021).

Nevertheless, EFSA is of the opinion that the design of the field trials is in accordance with the expected agricultural practices. To justify this opinion, EFSA should have provided a much more detailed reasoning. 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 are fulfilled. In light of the information available, we assume that the application and the data provided do not sufficiently represent the bio-regional conditions under which these plants are likely to be grown.

No experiments were requested to show to which extent specific environmental conditions influence plant composition and agronomic characteristics. Hence, no data were made available as requested in Implementing regulation 503/2013 to assess whether the expected environmental conditions under which the plants are likely to be cultivated will influence the expression of the studied endpoints.

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

Only 9 agronomic and phenotypic endpoints were subjected to statistical analysis, 6 of them were significantly different (days to flowering, plant height, days to maturity, lodging, final stand count and seed weight). This high number of significant findings should have prompted further investigations.

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, it appears that the data on gene expression were confined to a single variety. Therefore, EFSA should also have requested additional data from transgenic maize varieties that are, for example, cultivated in South America.

However, EFSA has not taken 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 of the genetic backgrounds on gene expression as requested in EU Regulation 503/2013.

Data from compositional analysis show the need for further investigations

63 constituents were subjected to statistical analysis. The statistical results show that 32 analytes were significantly different between the GM maize MON95379 and its conventional counterpart.

This means that >50% of the assessed endpoints are significantly different. According to experts from Member States, “significant results obtained by chance alone is 10%, considering that the conventional counterpart is an isogenic non-GM line and 90% confidence intervals are applied. Due to the high number of significant differences, it is recommended that all significances observed between the GM crop, its conventional counterpart and any other test material (including Type 2 analytes) are discussed individually.”

Given the above reasoning on the impact of environmental factors and genetic backgrounds, as well as a higher number of significant findings, EFSA should indeed 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, 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 factors 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 genetic backgrounds.

However, only samples from field sites located in the US were used to generate the data, and the impact of environmental factors were not assessed in detail. Only one transgenic variety was grown in the field trials.

Consequently, the data presented by the applicant and accepted by EFSA are insufficient to conclude on the impact of environmental factors or different genetic backgrounds on plant composition and agronomic characteristics.

Based on the available data, no final conclusions can be drawn on the safety of the plants. Therefore, the data neither fulfill the requirements of Implementing Regulation 503/2013 nor Regulation

1829/2003. This is also underlined by several statements made by experts from Member States (EFSA, 2022b).

In summary, the GE maize plants tested in the field trials do not sufficiently represent the products intended for import.

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;”*

Findings from molecular characterisation and comparative approach

As explained above, many significant changes were identified: more than half of the parameters measured for agronomic characteristics and plant composition were significantly different. Even if the changes taken as isolated data might not directly raise safety concerns, the overall number of effects should have been considered as a starting point for much more detailed investigation into their potential health impacts.

However, the data presented by the applicant did not take into account cultivation of the maize under more extreme drought conditions, i.e. neither under realistic agricultural conditions nor considering all relevant countries of cultivation. The range of differences and their significance are likely to be substantially increased in these conditions. Thus, without more data, the true range of unintended effects cannot be determined and safety cannot be demonstrated as requested by EU regulation.

Findings from a 90-day feeding study

A 90-day subchronic toxicity study was conducted by the applicant. According to EFSA, “no adverse effects were observed in rats in this 90-day toxicity study given diets containing maize MON 95379 up to 50% incorporation rate.”

However, Member States experts voiced several concerns about the study (EFSA, 2022b):

- a high variability was observed for several (significant different) endpoints, which indicates difficulties with study management and execution of this feeding experiment, and suggests that the results do not provide a reliable basis for drawing valid conclusions regarding the safety of GM maize MON95379.
- the power calculation presented by the applicant is not valid. This calculation was in fact only carried out for eight parameters, and the effect sizes selected by the applicant without substantiation (e.g. 200% for cholesterol, 100% for alkaline phosphatase or 50% for

creatinine) are not considered to be appropriate.

EFSA response to the concerns remain unsatisfactory. The Agency simply states that “the design of the 90-day feeding study was considered adequate” and “the outcome of the statistical analysis were considered adequate by the EFSA GMO Panel”. However, a critical assessment of the experts’ questions is missing.

Toxicity of Bt toxins

There is new evidence (currently undergoing final peer review) that the toxicity of Cry1A proteins needs to be reassessed: Jneid et al (2022) show that Cry1A toxins induce enterocyte death and intestinal stem cell (ISC) proliferation in the midgut of *Drosophila melanogaster*, a species that is supposed to be non-susceptible to these Bt toxins.

According to their research, after exposure, a high proportion of the ISC daughter cells differentiated into enteroendocrine cells instead of their initial enterocyte destiny. They show that that Cry1A toxins weaken the Cadherin-dependent adherens junction between the ISC and its immediate daughter progenitor, leading the latter to adopt an enteroendocrine fate. Hence, though not lethal to non-susceptible organisms, Cry toxins can interfere with conserved cell adhesion mechanisms, thereby disrupting intestinal homeostasis and enteroendocrine functions.

As the mechanisms of intestinal progenitor fate choice are conserved in the animal kingdom, it is crucial that the risk assessment of Cry1A toxins investigates whether these proteins can also promote an increased number of enteroendocrine cells (EECs) in other organisms (such as vertebrates and invertebrates). EECs, through the production of neuropeptides and hormones, are involved in the regulation of many physiological functions, such as feeding behavior, metabolism and immune response. Consequences of this increase in EEC number could be, for example, metabolic dysfunctions or inflammatory pathologies. More studies are needed to understand the physiological impacts of this change in intestinal cellular composition on organismal health.

EFSA assumes that the gastrointestinal tract of mammals, including humans, lacks receptors with high specific affinity to Cry proteins. However, for more than a decade they have ignored that there are further mechanisms and processes that may render Bt toxins biologically active in so-called ‘non-target’ organisms. These toxins are produced by bacteria which, in terms of taxonomy, belong to a group of *Bacillus cereus* which is known to produce many diseases in humans and animals. Cry1A toxins, in particular, are under discussion as to whether they cause severe effects, including in combination with other stressors or several Bt toxins: Grisolia et al. (2008) report embryo toxicity and developmental delay of mixtures of Cry1Aa + Cry1Ac and Cry1 Aa + Cry2A.

Therefore, the safety of the toxin and their combinations can no longer be generally assumed but has to be demonstrated before any further transgenic plants producing these toxins enter the market. It should also be taken into account that interactions with plant constituents, such as proteinase inhibitors, can multiply the toxicity of these toxins (MacIntosh et al., 1990).

It seems that a systematic literature review on the toxicity of Cry toxins as produced in the plants is crucial. Since it is a legal obligation, EFSA and Pioneer cannot escape this crucial step in risk assessment just because they presumed safety of the toxins before the risk assessment was conducted.

In any case, in regard to toxicology and potential synergistic or other combinatorial effects, the negative impacts of Bt toxins on human and animal health cannot be excluded a priori. Bt toxins have several modes of action. They are produced in the plants but their biological characteristics are altered and not identical to their natural templates (Hilbeck & Otto, 2015).

Several publications describe the effects of Bt toxins in mammals: some Cry toxins are known to bind to epithelial cells in the intestines of mice (Vázquez-Padrón et al., 1999, Vázquez-Padrón et al., 2000). As far as potential effects on health are concerned, Thomas and Ellar (1983), Shimada et al. (2003) Huffmann et al. (2004), Ito et al. (2004), Mesnage et al. (2013) and Bondzio et al. (2013) show that Cry proteins could potentially have an impact on the health of mammals. Further publications (de Souza Freire et al., 2014; Mezzomo et al., 2014) confirm hematotoxicity of several Cry toxins, including those being used in genetically engineered plants, such as Cry1Ab and Cry1Ac. These effects seem to occur after high concentrations and tend to become stronger after several days. Such observations call for the study of effects after long-term exposure to various dosages, including in combination with material sprayed with the complementary herbicides. In this context, it is important to consider that the stacked maize is also resistant to the herbicides glyphosate and glufosinate, and the resulting residues should be seen as potential co-stressors at the stage of consumption (see also Then & Bauer-Panskus, 2017).

Relevant findings show that the selectivity and efficacy of Bt toxins produced in GE plants can be influenced by many co-factors (see, for example, Then, 2010; Hilbeck & Otto, 2015). Higher toxicity can also cause lower selectivity (Then, 2010): if synergistic or additive effects occur that increase efficacy of the Bt toxin, its selectivity may be decreased and a wider range of non-target organisms may become susceptible.

One crucial impact factor in this context are protease inhibitors (PI), which show synergistic effects with Bt toxins, strongly enhancing their toxicity. It is likely that PI delay the degradation of Bt proteins and thereby also enhance their toxicity. In many of its comments on EFSA opinions, Testbiotech has highlighted these effects by referring, for example, to Pardo-López et al. (2009). However, EFSA has never provided a detailed response.

Testbiotech is aware of several publications confirming this gap in risk assessment that EFSA has constantly ignored or denied: as Monsanto already showed in the 1990s, maize, cotton and soybeans produce protease inhibitors (PI), which considerably enhance the toxicity of Bt proteins in plants. In the presence of PIs, Bt toxin will degrade much more slowly than in isolation. This results in a much higher toxicity of the Bt toxin (if it is taken up together with the plant tissue) compared to the isolated toxin (MacIntosh et al., 1990; Zhao et al., 1999; Zhang et al., 2000; Gujar et al., 2004; Zhu et al., 2007; Pardo-López et al., 2009; Ma et al., 2013; Mesén-Porras et al., 2020). The effects described indicate, for example, a 20-fold higher toxicity of Bt proteins if produced in the plants and taken up with PIs (MacIntosh et al., 1990).

It also should be taken into account that the toxicity of Bt toxins can not only be enhanced through interaction with plant enzymes such as PI, but also by Bt toxins (Sharma et al., 2004; Sharma et al., 2010; 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 co-stressors (Kramarz et al., 2007; Kramarz et al., 2009; Khalique and Ahmed, 2005; Singh et al., 2007; Zhu et al., 2005; Mason et al., 2011; Reardon et al., 2004; Nawrot-Esposito et al., 2020).

Therefore, any risk assessment that does not take synergistic effects caused by the combination of plant material or other stressors with the Bt toxin into account, is not reliable and systematically underestimates the risks (see also Testbiotech, 2021).

These issues are especially relevant for events expressing several Bt toxins, as the overall concentration of Bt toxins is higher and combinatorial effects with other stressors (such as residues from spraying) more likely.

However, as there is no data on interaction of the new Bt toxins there is a clear lack of data.

However, EFSA simply states that it believes that there are no interactions between the two Cry proteins.

Immunogenicity of the Bt toxins

There are several studies indicating that immune responses in mammals can be triggered by Bt toxins and have to be considered in this context. Studies with the Cry1Ac toxin (Moreno-Fierros et al., 2000; Vázquez-Padrón 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). Since Cry1Ac is also used as an adjuvant in vaccines, the risks to food consumption can be promoted through synergistic effects, this needs to be addressed and carefully examined.

The synergistic effects described by MacIntosh et al. (1990), Zhao et al. (1999), Zhang et al. (2000) Gujar et al. (2004), Zhu et al. (2007), Pardo-López et al. (2009), Ma et al. (2013), Mesén-Porras et al. (2020) causing higher toxicity of the Bt toxins are also relevant in risk assessment in regard to the immune system: combination with protease inhibitors is likely to be associated with a delay in the degradation of the Bt toxins after consumption. This delay in degradation extends the exposure of the intestinal immune system to Bt toxins and may trigger or enhance chronic inflammation and other immune responses (see also Testbiotech, 2021).

There are also findings from several whole food and feed studies, indicating a risk of inflammatory processes caused by maize producing Bt toxins: A study testing corn with a combination of Bt toxins (Cry1Ab and Cry34Ab1) indicates inflammation in rats (Zdziarski et al., 2018), as well as a study by Carman et al. (2013), feeding a triple stack of NK603, MON863 and MON810 maize. In addition, Ibrahim & Okasha (2016) found indication of inflammatory processes in the jejunum of rats fed with MON810 maize.

In this context, it is relevant that Bt toxins produced by plants can survive digestion to a much higher degree than has been assumed by EFSA and shown by the data of the applicant. 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; therefore, there is enough time for interaction between various food compounds.

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.

These issues are especially relevant for events expressing several Bt toxins, as the overall concentration of Bt toxins is higher compared to the parental plants.

Not only is the concentration of Bt toxins higher in the stacked Maize, there is also a higher likelihood of combinatorial effects with other stressors. However, neither EFSA nor the applicant considered the potential enhancement of toxic or immunogenic effects caused by interaction with plant components such as PI. In this context, potential impacts on the microbiome also have to be taken into account (see below).

EFSA (2022a), states “To date, there is no evidence for adjuvanticity in the GMOs assessed by the Panel.” However, this statement upends the legal requirements. In accordance with the precautionary principle, EU regulation requests that the safety of GMOs is demonstrated prior to applying for import. Since it cannot generally be denied that Cry toxins have adjuvant effects, it is up to the applicant to provide evidence that these effects are absent in the stacked maize. Therefore, EFSA cannot simply take a different approach and wait for evidence of adjuvant effects to appear.

Allergenicity

EFSA assessment of allergenic risks (EFSA, 2022d) is not based on a sufficiently realistic exposure to newly introduced proteins and their interactions. Different routes of exposure, the timing of exposure, microbial exposure, oral and gut microbiota composition, epithelial barrier integrity and/or non-allergenic components of the food matrix, such as immune-modulating components (adjuvants) of allergenic sources that facilitate immune responses, all have to be considered. In particular, the high number of proteins additionally expressed in the plants make it essential for appropriate data to be made available.

However, the necessary methodology is neither provided nor requested by EFSA. Therefore, the outcome of assessing allergenicity cannot be regarded as being sufficient.

5. 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. Maize volunteers can be found in the EU on a regular basis as has been reported from Palau-del-màs et al. (2009) in Spain or from Pascher (2016) in Austria. Further, in awareness of the biological characteristics of the GE 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. Under these circumstances, even a rare single outcrossing that goes unnoticed can have a huge long-term impact on the agro-ecosystems.

Furthermore, the EFSA (2022a) 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.
- Furthermore, 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 (Bauer-Panskus et al., 2020). This issue is relevant for gene flow from maize to as well from teosinte to maize. The

consideration of possible next generation effects is still absent in the latest EFSA statement (2022c) regarding the teosinte situation in France and Spain.

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.

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 (see also comments from Member States experts, EFSA, 2022b).

In addition, the example of maize MON 95379 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 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. *Environmental Sciences Europe*, 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 in Food Science & Technology*, 120: 325-337. <https://doi.org/10.1016/j.tifs.2022.01.002>
- Bøhn, T. (2018) Criticism of EFSA's scientific opinion on combinatorial effects of ‘stacked’ GM plants. *Food Chem. Toxicol*, 111: 268-274. <https://doi.org/10.1016/j.fct.2017.11.023>
- Bøhn, T., Rover, C.M., Semenchuk, P.R. (2016) *Daphnia magna* negatively affected by chronic exposure to purified Cry-toxins. *Food Chem. Toxicol.* 91: 130-140. <https://doi.org/10.1016/j.fct.2016.03.009>
- Bondzio, A., Lodemann, U., Weise, C., Einspanier, R. (2013) Cry1Ab treatment has no effects on viability of cultured porcine intestinal cells, but triggers hsp70 expression. *PloS One*, 8: e67079. <https://doi.org/10.1371/journal.pone.0067079>
- Broderick, N.A., Robinson, C.J., McMahon, M.D., Holt, J., Handelsman, J., Raffa, K.F. (2009) Contributions of gut bacteria to *Bacillus thuringiensis*-induced mortality vary across a range of Lepidoptera. *BMC Biol*, 7: 11. <https://doi.org/10.1186/1741-7007-7-11>
- Carman, J. A., Vlieger, H. R., Ver Steeg, L. J., Sneller, V. E., Robinson, G. W., Clinch-Jones, C. A., ... & Edwards, J. W. (2013). A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet. *J Org Syst*, 8(1), 38-54. <http://www.organic-systems.org/journal/81/8106.pdf>
- Cesco, S., Lucini, L., Miras-Moreno, B., Borruso, L., Mimmo, T., Pii, Y., ... & Trevisan, M. (2021) The hidden effects of agrochemicals on plant metabolism and root-associated microorganisms. *Plant Science*, 311: 111012. <https://doi.org/10.1016/j.plantsci.2021.111012>

Chowdhury, E.H., Kuribara, H., Hino, A., Sultana, P., Mikami, O., Shimada, N., Guruge, K.S., Saito, S., Nakajima, Y. (2003) Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. *J Anim Sci*, 81(10): 2546-2551. <https://doi.org/10.2527/2003.81102546x>

Commission Committee for the Common Organisation of Agricultural Markets (2021) EU Cereals Trade 2021/22, Marketing Year July – September, 26 November 2021). <https://circabc.europa.eu/sd/a/a1135630-e8e9-4531-a522-23670f75e2c5/cereals-trade-2017-18-marketing-year-july-december.pdf>

de Souza Freire, I., Miranda-Vilela, A.L., Barbosa, L.C.P., Martins, E.S., Monnerat, R.G., Grisolia, C.K. (2014) Evaluation of cytotoxicity, genotoxicity and hematotoxicity of the recombinant spore-crystal complexes Cry1Ia, Cry10Aa and Cry1Ba6 from *Bacillus thuringiensis* in Swiss mice. *Toxins*, 6: 2872-2885. <https://doi.org/10.3390/toxins6102872>

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. *Journal of Hazardous Materials*, 389: 122152. <https://doi.org/10.1016/j.jhazmat.2020.122152>

EFSA (2019) Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. *EFSA Journal*, 17(3): 5634. <https://doi.org/10.2903/j.efsa.2019.5634>

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 (2022a) Scientific Opinion on the assessment of genetically modified maize MON 95379 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2020-170). *EFSA J*, 20(11): 7588. <https://doi.org/10.2903/j.efsa.2022.7588>

EFSA (2022b) Comments and opinions submitted by Member States during the three-month consultation period. OpenEFSA portal. <https://open.efsa.europa.eu/>

EFSA (2022c) 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 (2022d) Scientific Opinion on development needs for the allergenicity and protein safety assessment of food and feed products derived from biotechnology. *EFSA J*, 20(1): 7044. <https://doi.org/10.2903/j.efsa.2022.7044>

González-González, E., García-Hernández, A.L., Flores-Mejía, R., López-Santiago, R., Moreno-Fierros, L. (2015) The protoxin Cry1Ac of *Bacillus thuringiensis* improves the protection conferred by intranasal immunization with *Brucella abortus* RB51 in a mouse model. *Vet Microbiol*, 175(2-4): 382-388. <https://doi.org/10.1016/j.vetmic.2014.11.021>

- Grisolia, C. K., Oliveira, R., Domingues, I., Oliveira-Filho, E. C., Monerat, R. G., & Soares, A. M. (2009) Genotoxic evaluation of different δ -endotoxins from *Bacillus thuringiensis* on zebrafish adults and development in early life stages. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 672(2), 119-123. <https://doi.org/10.1016/j.mrgentox.2008.10.017>
- Guerrero, G.G., Dean, D.H., Moreno-Fierros, L. (2004) Structural implication of the induced immune response by *Bacillus thuringiensis* cry proteins: role of the N-terminal region. *Mol Immunol*, 41(12): 1177-1183. <https://doi.org/10.1016/j.molimm.2004.06.026>
- Guerrero, G.G. & Moreno-Fierros, L. (2007) Carrier potential properties of *Bacillus thuringiensis* Cry1A toxins for a diphtheria toxin epitope. *Scand J Immunol*, 66(6): 610-618. <https://doi.org/10.1111/j.1365-3083.2007.01992.x>
- Gujar, T., Kalia, V., Kumari, A., Prasad, T.V. (2004) Potentiation of insecticidal activity of *Bacillus thuringiensis* subsp. *kurstaki* HD-1 by proteinase inhibitors in the American bollworm, *Helicoverpa armigera* (Hübner). *Indian J Exp Biol*, 42: 157-163. <http://hdl.handle.net/123456789/23352>
- Hilbeck, A. & Otto, M. (2015) Specificity and combinatorial effects of *Bacillus thuringiensis* Cry toxins in the context of GMO risk assessment. *Front Environ Sci*, 3: 71. <https://doi.org/10.3389/fenvs.2015.00071>
- Huffmann, D.L., Abrami, L., Sasik, R., Corbeil, J., van der Goot, G., Aroian, R.V. (2004) Mitogenactivated protein kinase pathways defend against bacterial pore-forming toxins. *PNAS USA*, 101: 10995-11000. <https://doi.org/10.1073/pnas.0404073101>
- Ibarra-Moreno, S., García-Hernández, A.L., Moreno-Fierros L. (2014) Coadministration of protoxin Cry1Ac from *Bacillus thuringiensis* with metacestode extract confers protective immunity to murine cysticercosis. *Parasite Immunol*, 36(6): 266-270. <https://doi.org/10.1111/pim.12103>
- Ibrahim, M.A., & Okasha, E.F. (2016). Effect of genetically modified corn on the jejunal mucosa of adult male albino rat. *Exp Tox Pathol*, 68(10): 579-588. <https://doi.org/10.1016/j.etp.2016.10.001>
- Ito, A., Sasaguri, Y., Kitada, S., Kusaka, Y., Kuwano, K., Masutomi, K., Mizuki, E., Akao, T., Ohba, M. (2004) *Bacillus thuringiensis* crystal protein with selective cytotoxic action on human cells. *J Biol Chem*, 279: 21282-21286. <https://doi.org/10.1074/jbc.M401881200>
- Jarillo-Luna, A., Moreno-Fierros L., Campos-Rodríguez R., Rodríguez-Monroy, M.A., Lara-Padilla, E., Rojas-Hernández, S. (2008) Intranasal immunization with *Naegleria fowleri* lysates and Cry1Ac induces metaplasia in the olfactory epithelium and increases IgA secretion. *Parasite Immunol*, 30(1): 31-38. <https://doi.org/10.1111/j.1365-3024.2007.00999.x>
- Jneid, R., Loudhaief, R., Zucchini-Pascal, N., Nawrot-Esposito, M. P., Fichant, A., Rousset, R., ... & Gallet, A. (2022). *Bacillus thuringiensis* Cry1A toxins divert progenitor cell fate toward enteroendocrine lineage by diminishing cell adhesion with intestinal stem cells. *BioRxiv*. <https://doi.org/10.1101/2022.04.13.488147>

Khalique, F. & Ahmed, K. (2005) Compatibility of bio-insecticide with chemical insecticide for management of *Helicoverpa armigera* Huebner. Pak J Biol Sci, 8: 475-478. <https://dx.doi.org/10.3923/pjbs.2005.475.478>

Kramarz, P., de Vaufleury, A., Gimbert, F., Cortet, J., Tabone, E., Andersen, M.N., Krogh, P.H. (2009) Effects of Bt-maize material on the life cycle of the land snail *Cantareus aspersus*. Appl Soil Ecol, 42: 236-242. <https://doi.org/10.1016/j.apsoil.2009.04.007>

Kramarz, P.E., de Vaufleury, A., Zygmunt, P.M.S., Verdun, C. (2007) Increased response to cadmium and *Bacillus thuringiensis* maize toxicity in the snail *Helix aspersa* infected by the nematode *Phasmarhabditis hermaphrodita*. Environ Toxicol Chem, 26: 73-79. <https://doi.org/10.1897/06-095R.1>

Legorreta-Herrera, M., Oviedo Meza, R., Moreno-Fierros L. (2010) Pretreatment with Cry1Ac protoxin modulates the immune response, and increases the survival of plasmodium -infected CBA/Ca mice. BioMed Research International: 198921. <https://doi.org/10.1155/2010/198921>

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>

Ma, Y., Zhang, Y., Chen, R.-R., Ren, X.-L., Wan, P.-J., Mu, L.-L., Li, G.-Q. (2013) Combined effects of three crystalline toxins from *Bacillus thuringiensis* with seven proteinase inhibitors on beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). Pestic Biochem Physiol, 105: 169-176. <https://doi.org/10.1016/j.pestbp.2013.01.007>

Mason, K.L., Stepien, T.A., Blum, J.E., Holt, J.F., Labbe, N.H., Rush, J.S., Raffa, K.F., Handelsman, J. (2011) From commensal to pathogen: translocation of *Enterococcus faecalis* from the midgut to the hemocoel of *Manduca sexta*. mBio 2: e00065-00011. <https://doi.org/10.1128/mBio.00065-11>

Mesén-Porras, E., Dahdouh-Cabia, S., Jimenez-Quiros, C., Mora-Castro, R., Rodríguez, C., Pinto-Tomás, A. (2020) Soybean protease inhibitors increase *Bacillus thuringiensis* subs. *israelensis* toxicity against *Hypothenemus hampei*. Agronomía Mesoamericana, 31: 461-478. <https://doi.org/10.15517/am.v31i2.36573>

Mesnage, R., Clair, E., Gress, S., Then, C., Székács, A., & Séralini, G. E. (2013). Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. Journal of Applied Toxicology, 33(7), 695-699. <https://doi.org/10.1002/jat.2712>

Mezzomo, B.P. (2013) Hematototoxicity of *Bacillus thuringiensis* as spore-crystal strains Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa in Swiss albino mice. J Hematol Thromb Dis, 1(1): 1-9. <http://repositorio.unb.br/handle/10482/18532>

- Moreno-Fierros, L., García, N., Gutiérrez, R., López-Revilla, R., Vázquez-Padrón, R.I. (2000) Intranasal, rectal and intraperitoneal immunization with protoxin Cry1Ac from *Bacillus thuringiensis* induces compartmentalized serum, intestinal, vaginal and pulmonary immune responses in Balb/c mice. *Microbes and Infection*, 2(8): 885-890. [https://doi.org/10.1016/S1286-4579\(00\)00398-1](https://doi.org/10.1016/S1286-4579(00)00398-1)
- Moreno-Fierros, L., García-Hernández, A.L., Ilhuicatzí-Alvarado, D., Rivera-Santiago, L., Torres-Martínez, M., Rubio-Infante, N., Legorreta-Herrera, M. (2013) Cry1Ac protoxin from *Bacillus thuringiensis* promotes macrophage activation by upregulating CD80 and CD86 and by inducing IL-6, MCP-1 and TNF- α cytokines. *International Immunopharmacology*, 17(4): 1051-1066. <https://doi.org/10.1016/j.intimp.2013.10.005>
- Nawrot-Esposito, M. P., Babin, A., Pasco, M., Poirié, M., Gatti, J. L., & Gallet, A. (2020). *Bacillus thuringiensis* bioinsecticides induce developmental defects in non-target *Drosophila melanogaster* larvae. *Insects*, 11(10), 697. <https://doi.org/10.3390/insects11100697>
- 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. *Environmental Sciences Europe*, 28(1):28-30. <https://doi.org/10.1186/s12302-016-0098-1>
- 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 Research*, 18(4): 583-594. <https://doi.org/10.1007/s11248-009-9250-7>
- Reardon, B.J., Hellmich, R.L., Sumerford, D.V., Lewis, L.C. (2004) Growth, Development, and Survival of *Nosema pyrausta* -Infected European Corn Borers (Lepidoptera: Crambidae) Reared on Meridic Diet and Cry1Ab. *J Econ Entomol*, 97: 1198-1201. <https://doi.org/10.1093/jee/97.4.1198>
- Rubio-Infante, N. & Moreno-Fierros, L. (2016) An overview of the safety and biological effects of *Bacillus thuringiensis* Cry toxins in mammals. *J Appl Toxicol*, 36(5): 630-648. <https://doi.org/10.1002/jat.3252>
- Rubio-Infante, N., Ilhuicatzí-Alvarado, D., Torres-Martínez, M., Reyes-Grajeda, J.P., Nava-Acosta, R., González-González, E., Moreno-Fierros, L. (2018) The macrophage activation induced by *Bacillus thuringiensis* Cry1Ac protoxin involves ERK1/2 and p38 pathways and the interaction with cell-Surface-HSP70. *J Cell Biochem*, 119(1): 580-598. <https://doi.org/10.1002/jcb.26216>
- Sharma, H.C., Sharma, K.K., Crouch, J.H. (2004) Genetic transformation of crops for insect resistance: potential and limitations. *Crit Rev Plant Sci*, 23(1): 47-72. <https://doi.org/10.1080/07352680490273400>

Sharma, P., Nain, V., Lakhanpaul, S., Kumar, P.A. (2010) Synergistic activity between *Bacillus thuringiensis* Cry1Ab and Cry1Ac toxins against maize stem borer (*Chilo partellus* Swinhoe). *Lett Appl Microbiol*, 51(1):42-47. <https://doi.org/10.1111/j.1472-765X.2010.02856.x>

Shimada, N., Kim, Y.S., Miyamoto, K., Yoshioka, M., Murata, H. (2003) Effects of *Bacillus thuringiensis* Cry1Ab toxin on mammalian cells. *J Vet Med Sci*, 65(2): 187-191. <https://doi.org/10.1292/jvms.65.187>

Singh, G., Rup, P.J., Koul, O. (2007) Acute, sublethal and combination effects of azadirachtin and *Bacillus thuringiensis* toxins on *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae. *Bull Entomol Res*, 97: 351-357. <https://doi.org/10.1017/S0007485307005019>

Tabashnik, B.E., Fabrick, J.A., Unnithan, G.C., Yelich, A.J., Masson, L., Zhang, J., Bravo, A., Soberón, M. (2013) Efficacy of genetically modified Bt toxins alone and in combinations against pink bollworm resistant to Cry1Ac and Cry2Ab. *PLOS ONE*, 8(11): e80496. <https://doi.org/10.1371/journal.pone.0080496>

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>

Then, C. (2010) Risk assessment of toxins derived from *Bacillus thuringiensis*: synergism, efficacy, and selectivity. *Environ Sci Pollut Res Int*, 17: 791-797. <https://doi.org/10.1007/s11356-009-0208-3>

Then, C., & Bauer-Panskus, A. (2017) Possible health impacts of Bt toxins and residues from spraying with complementary herbicides in genetically engineered soybeans and risk assessment as performed by the European Food Safety Authority EFSA. *Environ Sci Eur*, 29(1): 1. <https://doi.org/10.1186/s12302-016-0099-0>

Thomas, W.E. & Ellar, D.J. (1983) *Bacillus thuringiensis* var *israelensis* crystal delta-endotoxin: effects on insect and mammalian cells in vitro and in vivo. *J Cell Sci*, 60(1): 181-197. <https://jcs.biologists.org/content/60/1/181.short>

Trtikova, M., Wikmark, O.G., Zemp, N., et al. (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. *Scientific Reports*, 7:1560. <https://www.nature.com/articles/s41598-017-01478-w>

Tu, P., Gao, B., Chi, L., Lai, Y., Bian, X., Ru, H., & Lu, K. (2019) Subchronic low-dose 2, 4-D exposure changed plasma acylcarnitine levels and induced gut microbiome perturbations in mice. *Scientific reports*, 9(1), 1-11. <https://www.nature.com/articles/s41598-019-40776-3>

Vázquez-Padrón, R.I., Moreno-Fierros, L., Neri-Bazán, L., de la Riva, G.A., López-Revilla, R. (1999) Intragastric and intraperitoneal administration of Cry1Ac protoxin from *Bacillus thuringiensis* induces systemic and mucosal antibody responses in mice. *Life Sciences*, 64: 1897-1912. [https://doi.org/10.1016/S0024-3205\(99\)00136-8](https://doi.org/10.1016/S0024-3205(99)00136-8)

Vázquez-Padrón, R.I., Gonzáles-Cabrera, J., García-Tovar, C., Neri-Bazan, L., Lopéz-Revillac, L., Hernández, M., Moreno-Fierro, L., de la Riva, G.A. (2000) Cry1Ac protoxin from *Bacillus thuringiensis* sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. *Biochem Biophys Res Commun*, 271(1): 54-58. <https://doi.org/10.1006/bbrc.2000.2584>

Walsh, M.C., Buzoianu, S.G., Gardiner, G.E., Rea, M.C., Gelencsér, E., Jánosi, A., Jánosi, A., Epstein, M.M., Lawlor, P.G. (2011) Fate of transgenic DNA from orally administered Bt MON810 maize and effects on immune response and growth in pigs. *PLoS ONE*, 6(11): e27177. <https://doi.org/10.1371/journal.pone.0027177>

Zdziarski, I.M., Carman, J.A., Edwards, J.W. (2018) Histopathological investigation of the stomach of rats fed a 60% genetically modified corn diet. *Food Sci Nutr*, 9: 763-796. <https://doi.org/10.4236/fns.2018.96058>

Zhang, J., Wang, C., Qin, J. (2000) The interactions between soybean trypsin inhibitor and δ -endotoxin of *Bacillus thuringiensis* in *Helicoverpa armigera* larva. *J Invertebr Pathol*, 74(5): 259-266. <https://doi.org/10.1006/jipa.2000.4936>

Zhao, J.Z., Fan, Y.L., Fan, X.L., Shi, X.P., Lu, M.G. (1999) Evaluation of transgenic tobacco expressing two insecticidal genes to delay resistance development of *Helicoverpa armigera*. *Chin Sci Bull*, 44: 1871-1874. <https://doi.org/10.1007/BF02886343>

Zhu, Y.C., Abel, C.A., Chen, M.S. (2007) Interaction of Cry1Ac toxin (*Bacillus thuringiensis*) and proteinase inhibitors on the growth, development, and midgut proteinase activities of the bollworm, *Helicoverpa zea*. *Pestic Biochem Physiol*, 87(1): 39-46. <https://doi.org/10.1016/j.pestbp.2006.05.004>

Zhu, Y.C., Adamczyk, J.J., West, S. (2005) Avidin, a potential biopesticide and synergist to *Bacillus thuringiensis* toxins against field crop insects. *J Econ Entomol*, 98: 1566-1571. <https://doi.org/10.1093/jee/98.5.1566>