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Testbiotech comment on EFSA´s opinion on the assessment of genetically engineered soybean MON 87751 for food and feed uses under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2014–121) by company Monsanto

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Introduction

Soybean MON 87751 produces two insecticidal toxins: Cry1A.105 and Cry2Ab2. It is the second genetically engineered soybean to produce Bt toxins that was assessed by EFSA.

Soybeans are known to be one of the major allergenic food plants. Bt toxins are known for their potential to enhance immune responses to allergens; therefore assessment of impacts on health from soybeans that produce Bt toxins deserve special attention.

1. Molecular characterisation

The Bt toxins produced in the plants are not derived from naturally occurring *Bacillus thuringiensis*, but are synthesised the lab: Cry1A.105 is a chimeric protein with a mixture of elements stemming from Cry1Ab, Cry1F and Cry1Ac. It is meant to have a higher toxicity in pest insects compared to its natural precursors. Cry2Ab2 is a truncated and modified version of the naturally occurring Bt toxin.

The exact DNA sequence inserted in the plants has not been made publicly available; the flanking regions and open frames have likewise not been made available. This information is, however, highly relevant for risk assessment: both the assessment of the specific toxicity of the Bt proteins produced in the plants and the assessment of other gene products, such as miRNA, are dependent on this information. Therefore, these data should made publicly available.

Furthermore, 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 at five locations, without specific assessment of environmental interaction. Despite the relatively small set of data, findings indicate a significant range of variation in the content of the Bt toxins produced in the plants. This variation might be significantly increased if the event is introduced into other varieties, or if the plants are grown under different environmental conditions. As a result, the true range of variation in Bt toxin concentration remains unknown.

Further, the method used to determine the amount of Bt toxins (ELISA) is known to be dependent on the specific protocols used. The data are not sufficiently reliable without further evaluation by independent labs. For example, Shu et al. (2018) highlight difficulties in measuring the correct concentration of Bt toxins produced by the genetically engineered plants (see also Székács et al.,

2011). Without fully evaluated test methods to measure the expression and the concentration of the Bt toxins, risk assessment suffers from substantial methodological gaps.

There are also uncertainties in regard to the biochemical characteristics and the true toxicity of the Bt proteins; the proteins derived from bacteria used for safety testing were only comparable but not identical to the proteins produced in the plants.

Many more detailed investigations should have been carried out to examine changes in gene expression and unintended gene products, including the use of 'omics' techniques.

In summary, the data provided by the company cannot be regarded as conclusive.

2. Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)
Field trials for compositional and agronomic assessment of the soybeans were conducted in the US for only one year; at 8 locations for compositional analysis and 9 locations for agronomic characteristics. These trials were not carried out in any other soybean growing region such as Brazil and Argentina.

In addition to the true comparator plants, which are supposed to have an isogenic background, a high number of additional reference varieties were used (19 for compositional analysis and 20 for agronomic characteristics). These additional reference groups create a large set of data that can mask the relevant differences between the genetically engineered plants and their true comparator.

A low number of agronomic characteristics (nine) were tested, around half of them (four) were found to be significantly different in comparison to the true comparator plants. They were, however, within the range of data derived from the large number of additional reference lines.

On compositional analysis, 16 datasets out of 74 were not used for statistical analysis. 14 of the remaining 52 datasets on seeds were found to be significantly different in comparison to the true comparator plants; they were within the range of data derived from the large number of additional reference lines. Only six criteria were used for conducting comparison on forage; half were found to be significantly different in comparison to the true comparator plants.

Taken as isolated data these differences might not directly raise safety concerns. Nevertheless, the large overall number of effects and the low number of criteria that were tested should have led to further investigations. Therefore, EFSA should have requested further studies e.g.

- data from omics (proteomics, transcriptomics, metabolomics),
- data representing more extreme environmental conditions such as those caused by climate change,
- data representing more areas of commercial soy cultivation,
- more data on stress reactions under controlled conditions and
- more criteria to be tested, including all parts from the plants.

In addition, more varieties carrying the transgenes should have been included in the field trials to examine how the gene constructs interact with the genetic background of the plants.

Based on the available data, no final conclusions can be drawn on the safety of the plants.

Toxicology
28-day oral repeated dose toxicity studies in mice were conducted with the isolated proteins, but not in combination. Several significant effects were described.

It is debatable whether these feeding trials provide any useful information at all, since the proteins used for the feeding study were not identical with those produced in the plants and exposure is not comparable to that under practical conditions.

A 90-day feeding trial with soybean MON88751 was conducted. In this feeding trial only one dosage of soybean (30 %) was included as part of the diet, instead of several dosages as requested by existing guidance. Nevertheless, EFSA accepted the data.

There are further flaws in the design of the study: the control and the test diets were not checked for contamination with other GMOs. Upon request from experts from member states, EFSA (2018b) explicitly stated that such controls would not be requested:

> *"Analyses for contamination of the test or control materials with other GM materials is not requested by GMO regulation."*

Thus, it is unclear if, and to which extent, the effects of MON88751 were tested in these feeding trials, or if relevant effects were masked, triggered or influenced by other genetically engineered plants present in the diets. This is a violation of scientific standards that cannot be accepted under regulation 1829/2003 which foresees the highest scientific standards.

EFSA identified soybean milk as the most relevant source of exposure for humans. However, the only test material used was toasted defatted soybean meal; no other processed food or feed was tested. Furthermore, the presence of the Bt proteins in the diet was not tested. Thus, it remains doubtful whether the diet was suitable to test potential impacts on health effects from MON87751.

Several differences were found between the test and the control group, including blood chemistry and weight of organs. These effects were relatively small but might indicate subtle changes in the health of the test groups that would become evident only after longer period of time. In the assessment of the data, EFSA overlooked that health impacts are not necessarily linked to histopathological findings but can, for example, also emerge from changes in the intestinal microbiome (see for example Mao et al., 2018). But changes in the intestinal microbiome were not investigated.

Interestingly, EFSA (2018a) rejected the data from a second 90-day feeding study because

> *"test and control material [were] stored for more than one year, without any check for stability."*

Thus, while EFSA (2018a) points out the flaws in the second feeding study, it is strange that it still considers the data from the other feeding study to be conclusive.

More detailed (e.g. using several dosages) and long-term feeding studies would be necessary to assess potential impacts on health. These studies should include -omics data from animals as well as detailed assessment of the impact on the microbiome. The need for more detailed assessment is underlined by publications showing that Bt toxins raise several questions in regard to feed and food safety:

(1) There are several partially diverging theories about the exact mode of action of the Bt toxins at

the molecular level (see Then, 2010; Hilbeck & Otto, 2015). Thus, it cannot be assumed a priori that the toxins are inert in regard to human and animal health as argued in risk assessment for food and feed carried out by Monsanto.

(2) There are further uncertainties regarding the specificity of Bt toxins (Venter and Bøhn, 2016). Changes in specificity may emerge from structural modifications performed to render higher efficacy (see Hilbeck and Schmidt, 2006).

(3) In addition, there are findings in mammalian species showing that Bt toxicity is a relevant topic for detailed health risk assessment: some Cry toxins are known to bind to epithelial cells in the intestines of mice (Vázquez-Padrón et al., 1999).

(4) As far as potential effects on health are concerned, several publications (Thomas and Ellar 1983; Shimada et al., 2003; Mesnage et al., 2013; Huffman et al., 2004; Bondzio et al., 2013) show that Cry proteins may indeed have an impact on the health of mammals. For example, de Souza Freire et al., (2014) confirm hematotoxicity of several Cry toxins. Some of these effects seem to occur where there are high concentrations and tend to become stronger over longer periods of time.

(5) Further, the toxicity of Bt toxins can be enhanced through interaction with other compounds, such as plant enzymes (Zhang et al., 2000, Zhu et al., 2007; Pardo-López et al., 2009), other Bt toxins (Sharma et al., 2004; Tabashnik et al., 2013; Bøhn et al. 2016, Bøhn 2018), gut bacteria (Broderick et al., 2009), residues from spraying with herbicides (Bøhn et al. 2016, Bøhn 2018) and other 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).

In this context, it is relevant that Bt toxins can persist in the gut to a much higher degree than has been assumed by EFSA. Chowdhury et al., (2003) and Walsh et al. (2011) have found that when pigs were fed with Bt maize, Cry1A proteins could frequently and successfully still be found in the colon of pigs at the end of the digestion process. This means that Bt toxins are not degraded quickly in the gut and can persist in larger amounts until digestion is completed; and that there is enough time for interaction between various food compounds. Especially in soybeans, compounds such as trypsin inhibitors, can delay the degradation of Bt toxins (Pardo-López et al., 2009) and can therefore cause higher exposure and render higher toxicity compared to experiments with the proteins in isolation. It has to be emphasised that the data presented on thermal or enzymatic degradation of the isolated proteins do not allow the assessment of the true persistence of the Bt toxins in the food chain.

Further, as far as the exposure of the food chain with Bt toxins is concerned, EFSA should have requested data on the overall combined exposure to Bt toxins caused by the introduction of Bt plants in the EU. Currently, there are already 30 events that produce Bt toxins authorised for import. The exposure stemming from these imports should have been added to that of soybean MON88751 to assess exposure in a much more realistic scenario.

Consequently, the toxicological assessment carried out by EFSA is not sufficient to show food and feed safety.

Allergenicity

In the case of maize Bt11 x MIR162 x 1507 x GA21 (EFSA 2018c), EFSA has admitted relevant uncertainties in regard to the immunogenic effects of the Cry proteins: EFSA stated that there is

"limited experimental evidence available". The Bt toxins (Cry1F and Cry1Ab) produced by the stacked maize are considered to be similar to those produced in MON88751.

But in the case of MON88751, EFSA claims that the relevant questions were previously assessed in 2008:

> *"The GMO Panel has previously evaluated the safety of the Cry1A.105 and Cry2Ab2 proteins in maize MON 89034 and no concerns on allergenicity were identified (EFSA, 2008).*

This statement not only displays scientific ignorance in regard to more recent publications (for an overview see, for example, Then & Bauer-Panskus, 2017; Rubio-Infante & Moreno-Fierros, 2015), it is also a violation of the case by case approach that is required by EU regulation: in the case of soybeans, allergenicity and especially adjuvant effects have to be assessed in more detail compared to maize. Furthermore, the statement is in contradiction to the fact that in 2017, EFSA started a tender to "*literature review on adjuvanticity/immunogenicity assessment of proteins*" explicitly mentioning Cry toxins. As the tender states:

> *"Adjuvants are substances that, when coadministered with an antigen increase the immune response to the antigen and therefore might increase the allergic response. Understanding the structure and mechanisms resulting in potential adjuvanticity/immunogenicity of (novel) proteins and the conditions (e.g. environment, exposure) upon which this potential will be expressed and/or (de)regulated is an area of great development and scientific debate. The information provided by this call for tender will be important for the EFSA GMO Panel to further discuss how to incorporate and streamline potential new strategies for the risk assessment of adjuvanticity/immunogenicity of (novel) proteins into the EFSA GMO Panel scientific opinions on applications."*

To the best of our knowledge, no outcome of this tender was ever published. However, the language and the scope of the tender clearly show that even in 2017 no final conclusion could be drawn on the immunogenic properties of Cry toxins.

The need for more detailed investigations in regard to potential immunogenic effects is also underlined in the minority opinion in the case of maize Bt11 x MIR162 x 1507 x GA21 (EFSA 2018c), which clearly expresses concern that immunogenic effects of these Cry toxins cannot be ruled out.

In regard to the immunogenic potential of soybean MON87751, the EFSA assessment is unacceptable. Contrary to what is stated by EFSA, the immunogenic properties of the Cry toxins produced in the soybean were not sufficiently assessed. The EFSA opinion indicates certainty and safety without this being based on sufficient evidence.

Others

According to Regulation (EU) No 503/2013, 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 soybeans imported into the EU,
ii) the ports and silos where shipments of the soybeans are unloaded,
iii) the processing plants where the soybean are transferred to,
iv) the amount of the soybeans used on farms for feed, and

v) transport routes of the soybeans.

Environmental risk assessment

EFSA (2018a) acknowledges that feral genetically engineered soybean plants might occur outside cultivation areas. As the opinion from experts of Member States show (EFSA 2018b), EFSA did not consider all relevant regions and climate conditions while assessing the potential persistence of these volunteer plants.

Furthermore, environmental exposure through organic waste material, by-products, sewage or faeces containing MON87751 during or after the production process, and during or after human or animal consumption should be assessed in much more detail (see also EFSA, 2018b). Evolutionary mechanisms led to the emergence of the natural Bt toxins in soil bacteria, therefore, no conclusion can be drawn on effects that might be caused if the synthetic proteins were to be produced by soil bacteria after gene transfer. Thus the assumptions of EFSA (2018a) have to be rejected.

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

Conclusions and recommendations

The opinion of EFSA has to be rejected.

References:

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://www.sciencedirect.com/science/article/pii/S0278691516300722

Bøhn, T. (2018) Criticism of EFSA's scientific opinion on combinatorial effects of 'stacked' GM plants. Food and Chemical Toxicology. https://www.sciencedirect.com/science/article/pii/S0278691517306907

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

Chowdhury, E.H., Kuribara, H., Hino, A., Sultana, P., Mikami, O., Shimada, N., ... & 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. Journal of animal science, 81(10): 2546-2551. https://academic.oup.com/jas/article-abstract/81/10/2546/4789819

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

EFSA (2008) Scientific Opinion of the Panel on Genetically Modified Organisms on application (Reference EFSA-GMO-NL-2007-37) for the placing on the market of the insect-resistant genetically modified maize MON 89034, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2008;6(12):909, 30 pp. https://doi.org/10.2903/j.efsa.2008.909

EFSA (2017) Tender: Literature review on adjuvanticity/immunogenicity assessment of proteins, Ref: NP/EFSA/GMO/2017/01, https://www.efsa.europa.eu/de/tenders/tender/170407

EFSA GMO Panel (2018a) Opinion on the assessment of genetically modified soybean MON 87751 for food and feed uses under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2014–121). EFSA Journal 2018;16(7):5346, 32 pp. https://doi.org/10.2903/j.efsa.2018.5346

EFSA GMO Panel (2018b) Comments from the experts of Member States on the scientific opinion on the assessment of genetically modified soybean MON 87751 for food and feed uses under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2014–121). Accessed via the register of EFSA, http://registerofquestions.efsa.europa.eu/roqFrontend/login?0

EFSA GMO Panel (2018c) Scientific opinion on the assessment of genetically modified maize Bt11 x MIR162 x 1507 x GA21 and three subcombinations independently of their origin, for food and feed uses under Regulation (EC) No 1829/2003 (application EFSA-GMO-DE-2010-86). EFSA Journal 2018;16(7):5309, 35 pp. https://doi.org/10.2903/j.efsa.2018.5309

Hilbeck, A., Otto, M. (2015) Specificity and Combinatorial Effects of Bacillus Thuringiensis Cry Toxins in the Context of GMO Environmental Risk Assessment. Front. Environ. Sci. 3. https://doi.org/10.3389/fenvs.2015.00071

Hilbeck, A., Schmidt, J.E.U. (2006) Another View on Bt Proteins – How Specific are They and What Else Might They Do? Biopesticides international, 2(1), 1-50.

Huffman, D.L., Abrami, L., Sasik, R., Corbeil, J., Goot, F.G. van der, Aroian, R.V. (2004) Mitogen-activated protein kinase pathways defend against bacterial pore-forming toxins. Proc. Natl. Acad. Sci. U. S. A. 101, 10995–11000. https://doi.org/10.1073/pnas.0404073101

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.

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://www.sciencedirect.com/science/article/pii/S0929139309000808

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://onlinelibrary.wiley.com/doi/abs/10.1897/06-095R.1

Mao, Q., Manservisi, F., Panzacchi, S., Mandrioli, D., Menghetti, I., Vornoli, A., Bua, L., Falcioni, L., Lesseur, C., Chen, J., Belpoggi, F., Hu, J. (2018) The Ramazzini Institute 13-week pilot study on glyphosate and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome, Environmental Health, 17:50. https://doi.org/10.1186/s12940-018-0394-x

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

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. J. Appl. Toxicol. 33, 695–699. https://doi.org/10.1002/jat.2712

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, Invertebrate Neuropeptides, IX 30: 589–595. https://doi.org/10.1016/j.peptides.2008.07.027

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. Journal of Applied Toxicology, 36(5): 630-648. http://onlinelibrary.wiley.com/doi/10.1002/jat.3252/full

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: 47-72. https://doi.org/10.1080/07352680490273400

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, 187–191. https://doi.org/10.1292/jvms.65.187

Shu, Y., Romeis, J., Meissle, M. (2018) No interactions of stacked Bt maize with the non-target aphid *Rhopalosiphum padi* and the spider mite *Tetranychus urticae*. Frontiers in Plant Science, 9: 39. https://www.frontiersin.org/articles/10.3389/fpls.2018.00039

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

Székács, A., Weiss, G., Quist, D., Takács, E., Darvas, B., Meier, M., Swain, T., Hilbeck, A. (2011) Interlaboratory comparison of Cry1Ab toxin quantification in MON 810 maize by ezyme-immunoassay. Food and Agricultural Immunology, 23(2): 99-121. www.tandfonline.com/doi/abs/10.1080/09540105.2011.604773

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: e80496.

Then C (2010) Risk assessment of toxins derived from Bacillus thuringiensis - synergism, efficacy, and selectivity. Environ Sci Pollut R 17(3):791–797. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0080496

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. Environmental Sciences Europe, 29(1): 1. https://enveurope.springeropen.com/articles/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: 181–197. http://jcs.biologists.org/content/60/1/181.short

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. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0123011

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 Sci. 64, 1897–1912. https://doi.org/10.1016/S0024-3205(99)00136-8

Venter, H.J., Bøhn, T. (2016) Interactions between Bt crops and aquatic ecosystems: A review. Environ. Toxicol. Chem. 35, 2891–2902. https://doi.org/10.1002/etc.3583

Walsh, M.C., Buzoianu, S.G., Gardiner, G.E., Rea, M.C., Gelencsér, E., Jánosi, A., ... & 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. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0027177

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. 75: 259-266. https://doi.org/10.1006/jipa.2000.4936

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

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 Phys 87(1): 39-46. https://www.sciencedirect.com/science/article/pii/S0048357506000848