

## maize-59122

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**Organisation: Farmers Association Futuragra**

**Country: Italy**

**Type: Non Profit Organisation**

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**a. Assessment:**

### **4. Conclusions and recommendations**

Italy has been facing a decrease in domestic maize production in the recent years. While a decade ago Italy's maize demand was satisfied by domestic production, in 2010 and 2011 the country had to import about 20% of its needs and in 2012 it is expected to arrive close to 40%. This very negative trend comes from increasing challenges and difficulties farmers are facing in addressing impact of climate changes, and in particular increasing pest damages, occurring in the Italian corn growing area. Apart the negative impact on national food security, these decreasing maize yields are heavily affecting economic sustainability of maize growing, undermining farmers income and their ability to stay on the market in the coming years. One of the major elements that contributed to development of this scenario was for sure the arrival in Italy of the WCR (First presence was reported in Veneto in 2000). Today diabrotica is present all over the maize growing area of the country, in all Regions in Northern Italy, and also in Lazio and Tuscany, with a density of insects well above the thresholds for economic damages to the crops (due to roots and silks feeding). Agronomic solutions for CRW management are focused on crop rotation (but this is hard to do in a highly specialized farming environment as Italy), together with heavy use of insecticides. In particular huge quantities of soil granulate insecticides are required to control larvae, but also timely application of sprays (close to the flowering period) against adults. Environmental impact of these solutions is significant on quality of ground water and on non-target organisms, and the national ban on use of neonicotinoids for seed treatment made the situation even worse. Environmental impact, economic loss and limited efficacy of the current strategy to manage presence of WCR in Italy clearly emerge when favorable conditions for CRW occur, like in 2009. That year, just in Lombardy, over 42.000ha and 777 farms were attacked, with 12.000ha of maize totally destroyed by this pest. Economic loss for farmers and for the whole local food chain was very huge. The current scenario, expected to be influenced by further spreading of infestation of WCR in Italy and by the impact of climate change, is urging the adoption of innovative solutions, including new GM seed products, to control this pest, ensuring environmental and economic sustainability, but also increased food safety.

We recommend the Commission to ensure a science based approach to this new product, in line with recent positive EFSA opinion.

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### **5. Others**

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**Organisation: Individual**

**Country: Romania**

**Type: Individual**

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**a. Assessment:**

**4. Conclusions and recommendations**

EFSA conclusions are correct and for the future of agriculture. Europeans have become more realistic and give up some absurdities. Americas win because of this, and Europeans losing chances. Europe imports large amounts of soy in America, but is not allowed to grow. Where is the equality of opportunity principle?

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**Organisation: Association**

**Country: Romania**

**Type: Association**

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**a. Assessment:**

**4. Conclusions and recommendations**

EFSA conclusions are correct and for the future of agriculture. Europeans have become more realistic and give up some absurdities. Americas win because of this, and Europeans losing chances. Europe imports large amounts of soy in America, but is not allowed to grow. Where is the equality of opportunity principle?

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**Organisation: Association**

**Country: Romania**

**Type: Association**

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**a. Assessment:**

**4. Conclusions and recommendations**

Agricultural Producers Association - APA Braila, represents the interests farmers in one of the most important agricultural areas of Romania.

APA Braila noted with satisfaction, the conclusions body European Scientific – EFSA, on the safety of consumption of humans and animals, 59122 maize, a genetically modified plant resistance to Diabrotica a very dangerous pest this species.

APA Braila appreciate this endorsement, as an important step in order fully accessible European farmers to biotechnology agriculture.

APA Braila waiting with great interest, completion of the procedures, to reintroduce the culture of genetically modified soya, step European farmers important contribution to ensuring vegetable protein necessary for Europe.

Chairman, Costica Macelaru

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

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**a. Assessment:**  
**Molecular characterisation**

Open reading frames were identified that can give rise to unintended gene products in the plants. The DNA construct is flanked by DNA that is known to be functional and involved in the plant metabolism. Given these findings, the impact of the additional DNA constructs on the regulation and activity of the plant genome should have been investigated much more in detail.

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**Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)**

Many significant differences in compositional analysis were observed in comparison with the plant's conventional counterparts. In the assessment of these findings, reference was made to historical data unrelated to the actual field trials such as the ILSI database (EFSA, 2007). Since it is not sufficiently clear under which specific conditions these additional historical data were generated, this kind of comparison inevitably contains major uncertainties.

Several statistically significant findings in agronomic parameters came to light in the 2004 European field trials (germination, plant height, ear height, final population). According to EFSA, the differences were not consistent in locations and over the years. The reason for this might be that these differences only emerge under particular environmental conditions. The differences should therefore trigger more detailed analyses.

Furthermore, there are no standardised protocols for these Bt toxins in order to achieve reliable results which can be reproduced by other laboratories. (see also Szekacs et al., 2011). The content of the additional proteins Cry34Ab1 produced in the plant is highly variable. This may indicate genetic instability and result in unexpected reactions to specific environmental conditions. Several investigations show that genetically engineered plants can exhibit unexpected reactions under stress conditions (see for example: Matthews et al., 2005). This can also impact on the Bt content in the plants (Then & Lorch, 2008). It is not clear how these plants and the expression rate of the newly introduced proteins will be influenced by more extreme weather conditions such as drought or other environmental factors.

It must be noted that the compositional assessment and expression data rely entirely on company data. So far, no independent data on composition or agronomic parameters regarding Maize 59122 have been published.

In the light of these uncertainties, further independent studies under controlled environmental conditions are necessary to gain a better understanding of the genome x environment interactions and the changes in composition and agronomic performances. Fully evaluated methods to measure the expression of the newly introduced gene constructs should be established. Further, the maize has only been grown in Spain, Bulgaria and Hungary and not in other regions of the EU, thus further data representing the true bioclimatic diversity within the EU are necessary.

EFSA (2007) Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-NL-2005-12) for the placing on the market of insect-resistant genetically modified maize 59122, for food and feed uses, import and processing under Regulation (EC) No 1829/2003, from Pioneer Hi-Bred International, Inc. and Mycogen Seeds, c/o Dow Agrosciences LLC, EFSA Journal, 470: 1–25.

Matthews, D., Jones, H., Gans, P., Coates, St. & Smith, L.M.J. (2005) Toxic secondary metabolite production in genetically modified potatoes in response to stress. *Journal of Agricultural and Food Chemistry*, 10.1021/jf050589r.

Székács, A., Weiss, G., Quist, D., Takács, E., Darvas, B., Meier, M., Swain, T., Hilbeck, A. (2011) Inter-laboratory comparison of CryIAb toxin quantification in MON 810 maize by enzyme-immunoassay, *Food and Agricultural Immunology*, 23(2): 99-121.

Then, C. & Lorch, A. (2008) A simple question in a complex environment: How much Bt toxin do genetically engineered MON810 maize plants actually produce?: in Breckling B, Reuter H, Verhoeven R (eds) (2008) *Implications of GM-Crop Cultivation at Large Spatial Scales.*, Theorie in der Ökologie 14. Frankfurt, Peter Lang.

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## **b. Food Safety Assessment: Toxicology**

The applicants performed two toxicological 90 day studies. In the first (Malley et al., 2007), contrary to OECD guidelines, only one dose level was used for the whole 90 day rat study. The study showed several differences in haematology and organ weights. „... male rats receiving the maize 59122 diet showed statistically significant decreases in absolute reticulocyte count and red cell distribution width as well as increases in mean corpuscular

haemoglobin and mean corpuscular haemoglobin concentration. Females showed an increase in platelet count.“ „Organ weight determinations revealed a statistically significant increase in uterus weight in females receiving the maize 59122 diet.“(EFSA 2007)

A more recent study (He et al., 2008), also found several haematological effects: „Statistical differences ( $p < 0.05$ ) were observed in certain hematology and serum chemistry response variables between rats consuming diets formulated with 59122 or 091 Control flour compared to AIN93G diet.“

The differences were attributed to the diet formula.

None of the feeding studies are based on acceptably scientific standards. For example, other genetically engineered plants in the diet (such as genetically engineered soy) are not excluded, thus relevant effects might be masked by these constituents in the diet used in the trials.

Further, it should be a matter of concern that both studies were performed by the company.

Detailed in vitro studies should have been performed on the possible toxicity of the Cry toxins in vertebrates. As some publications show (Soberon et al., 2009, Mesnage et al., 2012), there are mechanisms and findings in regard to potential toxicity in mammals. Since the imported maize will at the very least contain residues from spraying, possible interactivity with the Bt toxin should have been considered as well.

No long term feeding studies, including reproductive parameters were performed and the potential impact on wildlife was not considered at all. So far, no independent data regarding the food and feed safety of Maize 59122 have been published.

EFSA (2007) Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-NL-2005-12) for the placing on the market of insect-resistant genetically modified maize 59122, for food and feed uses, import and processing under Regulation (EC) No 1829/2003, from Pioneer Hi-Bred International, Inc. and Mycogen Seeds, c/o Dow Agrosciences LLC, EFSA Journal, 470: 1–25.

Malley, L.A., Everds, N.E., Reynolds, J., Mann, P.C., Lamb, I., Rood, T., et al. (2007) Subchronic feeding study of DAS-59122-7 maize grain in Sprague-Dawley rats. *Food and chemical toxicology*, 45(7): 1277-1292.

Mesnage, R., Clair, E., Gress, S., Then, C., Székács, A., Séralini, G.-E. (2012) Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide, *Journal of Applied Toxicology*, DOI: 10.1002/jat.2712.

He, X. Y., Huang, K. L., Li, X., Qin, W., Delaney, B., & Luo, Y. B. (2008). Comparison of grain from corn rootworm resistant transgenic DAS-59122-7 maize with non-transgenic maize grain in a 90-day feeding study in Sprague-Dawley rats. *Food and Chemical Toxicology*, 46(6), 1994-2002.

<http://www.sciencedirect.com/science/article/pii/S0278691508000628>.

Soberón, A., Gill, S.S., Bravo, A. (2009) Signaling versus punching hole: How do *Bacillus thuringiensis* toxins kill insect midgut cells? *Cell. Mol. Life Sci.* 66: 1337 – 1349.

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## Allergenicity

No empirical investigations were performed concerning allergies or other impacts on the immune system. The level of most relevant maize allergens was not determined. Adjuvant effects and impact on the immune system were not considered, despite it being known that bacterial proteins such as Bt can affect the immune system.

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### **Nutritional assessment**

According to EFSA, the studies as presented by the applicants indicate that feed produced from Maize 59122 is as nutritious and wholesome as other maize. However, all five studies that were assessed by EFSA were conducted by the applicants themselves. In general, no conclusions on the safety of the genetically engineered plants can be drawn from nutritional studies.

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### **3. Environmental risk assessment**

The mode of action of Bt toxins is not fully understood and is even a matter of controversial debate (Pigott & Ellar, 2007). Strict selectivity of the Bt toxins is not shown by empirical evidence but deduced from its mode of action as described previously.

It is known that the Bt toxins (or their combination) present in Maize 59122 are indeed less specific than supposed. As EFSA states: „The apparent activity of Cry34Ab1/Cry35Ab1 at high concentrations against the lepidopteran species (e.g., *Ostrinia nubilalis* and *Sitotroga cerealella*) was not expected based on the known spectrum of activity (Coleoptera only) of these binary proteins. The EFSA GMO Panel considers that there are indications of a potential hazard to Lepidoptera owing to cross-order activity at high Cry34Ab1/Cry35Ab1 protein concentrations.“

In this case, the specific mode of action and potential for synergistic toxicity is not known. Thus no conclusion can be reached upon the role of specific receptors in the gut or other factors that might impact toxicity. The most effective mixture of toxins is a further unknown factor. Any change in the proportions of the single toxins during the vegetation period might influence their actual toxicity. This was not taken into account in the risk assessment.

As Pardo Lopez et al. (2009) and Pigott et al. (2008) show, synthetically derived and modified Bt toxins can show much higher toxicity than native proteins. Even small changes in the structure of the proteins can cause huge changes in toxicity. Some plant enzymes that diminish the digestion of proteins (protease inhibitors) can strongly enhance the toxicity of Bt toxins (Pardo Lopez et al., 2009). Even the presence of very low levels of protease inhibitors can multiply the insecticidal activity of some Cry toxins. It is known that maize produces such inhibitors (Shulmina et al., 1985).

Synergistic effects can become highly problematic for non-target organisms. Interactivity between the toxins or in combination with environmental toxins, bacteria, plant enzymes or pesticides can cause unexpected higher toxicity and lower selectivity (Then, 2010). None of these potential synergies with other stressors was investigated during risk assessment – which is surprising since it is known that the toxicity of Cry34Ab1 and Cry35Abe is based on synergistic effects.

It also should be considered that the plants might not be marketed to be sprayed with the complementary herbicide (glufosinate). However, individual farmers might nevertheless apply the herbicide. Thus interactivity between the herbicide and the Bt toxins is definitely relevant for environmental risk assessment.

To assess the possible impact of maize 59122 on non-target organisms, tiered laboratory studies were conducted by the applicants. However, the studies presented by the applicants are of very poor quality. This was also remarked upon by several Member States during the consultation period. For example, the German Competent Authority criticises following points: „1.) Mainly microbial-derived toxin tests and not whole-plant tests were carried out, 2.) only a single ratio of the binary toxins was tested, 3.) the representativeness of the chosen experimental toxin ratios or concentrations remained unclear, 4.) effects were sometimes not statistically analysed, 5.) only short-term studies were conducted, 6.) often no sublethal effects were tested, 7.) not all relevant developmental stages of the non-target species were taken into account, 8.) no tri-trophic experiments were done, 9.) the relevance of species not native to Europe appears questionable, 10.) the amount of toxin uptake by non-targets was never recorded, 11.) no dose-effect relationship were established, 12.) whether the toxin was degraded during experimental period and/or the proof of bioactivity of the toxin was not recorded, 13.) only a single toxin dose was applied.“

EFSA also recognized the poor quality of the applicants' laboratory studies: „The EFSA GMO Panel notes that some of the lower-tier studies conducted by the applicant do not adhere to the general principles of good laboratory study design [ ... ], and therefore cannot be used to support the risk assessment.

However, instead of asking the applicants for more appropriate studies, EFSA sides with the applicants' opinion that cultivation of line 59122 poses a negligible risk to human health and the environment. In Testbiotech's opinion, this cannot be deduced from the applicants' studies because of a paucity of valid data. A comprehensive assessment would have been of huge importance because of the toxicological properties of Cry34Ab1/Cry35Ab1 proteins which differ from expectations. It was also stated by Member States that independent data regarding the possible environmental effects of Cry34/35Ab1 proteins are almost completely missing, as all studies are performed by or commissioned by the applicants.

For example, in the case of two relevant non-target organisms, ladybirds (Coccinellidae) and leaf beetles (Chrysomelidae), risk assessment shows severe deficiencies: Although only two studies on leaf beetles (one from Germany, one from Hungary, in which a member of the GMO Panel, Josef Kiss was involved and also voted on the opinion of EFSA) were considered, this was deemed sufficient to conclude on the occurrence of leaf beetles in maize fields all over Europe. Due to this premature assumption, most species were not tested at all. As for ladybirds, the authority in the Netherlands concluded that risks could not be excluded and that a case specific monitoring should be performed. In response EFSA simply asked the applicant, Dow AgroSciences, to perform further studies. Naturally the company was aware that a (costly) case specific monitoring might be requested and therefore had significant vested interest in the outcome of these investigations. In this case EFSA should have definitely asked for independent investigations. Instead, it was happy with the company's own studies and did not request further investigations or a case specific monitoring.

Even according to EFSA, there are no reliable data on the potential accumulation of the toxin in the soil. This deficiency cannot be replaced by monitoring after commercial cultivation but must be investigated and assessed before authorisation.

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.

Pigott, C.R. & Ellar, D.J. (2007) Role of Receptors in *Bacillus thuringiensis* Crystal Toxin Activity: *Microbiol Mol Biol Rev* 71 (2): 255–281.

Pigott, C.R., King, S.M., Ellar D.J. (2008) Investigating the Properties of *Bacillus thuringiensis* Cry Proteins with Novel Loop Replacements Created Using Combinatorial Molecular Biology. *Applied and Environmental Microbiology*: 3497–3511

Shulmina, A.I., Dronova, L.A., Shubin, V.V., et al. (1985) Determination of the chymotrypsin inhibitors, secondary structure of the chymotrypsin inhibitor from corn by the circular-dichroism method. *Biochemistry-Moscow*, 50(7): 980-982.

Then, C. (2010) Risk assessment of toxins derived from *Bacillus thuringiensis*-synergism, efficacy, and selectivity. *Environ Sci Pollut Res Int*; 17(3): 791-7.

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#### 4. Conclusions and recommendations

Maize 59122 cannot be considered safe for human and animal health or the environment. There are indications that its cultivation will lead to rise in Bt resistant insects. EFSA risk assessment must be rejected because it does not fulfill necessary scientific standards and therefore carries a level of uncertainty that is too high.

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#### 5. Others

##### (1) Resistance in pest insects

During the last few years, rootworm resistance against genetically engineered maize lines producing Cry3Bb1 has become wide spread in the USA. Literature shows that one of the main reasons for growing resistance is the fact that current crops do not follow the high dosage requirement in regard to western corn rootworm (WCR). According to literature, this is also true for Cry34/35Ab1 (see for example Tabashnik and Gould, 2012).

Accordingly, resistance development in rootworm feeding on Maize 59122 was as fast as it was in Bt maize expressing Cry3Bb1 (Lefko et al., 2008). This aspect is also acknowledged by EFSA: „Based on the available data, the EFSA GMO Panel concludes that WCR has the ability to evolve resistance to the Cry34Ab1/Cry35Ab1 proteins, especially if maize 59122 is used repeatedly and exclusively, and the WCR infestation levels are high.“

Further, according to EFSA, it looks like no fitness costs are related to the acquired resistance against the Cry34Ab1/Cry35Ab1 proteins – this is another indication that resistance in pest insects can become a severe problem. As Oswald et al (2012) indicate, the selection of pest insects with even higher fitness, cannot be excluded.

In response EFSA has proposed (amongst others things) a requirement for systematic crop rotation as risk mitigation where the Bt plants are grown. From the perspective of the risk



manager this is an interesting option: Since so far no rootworm are known in the EU that are resistant to crop rotation, the Commission could request adequate crop rotation in all regions where rootworm might become a problem in the maize fields. This would in effect render the cultivation of any Bt plants producing insecticides against the rootworm completely meaningless.

Lefko, S.A., Nowatzki, T.M., Thompson, S.D., Binning, R.R., Pascual, M.A., et al. (2008) Characterizing laboratory colonies of western corn rootworm (Coleoptera : Chrysomelidae) selected for survival on maize containing event DAS-59122-7. *J Appl Entomol* 132: 189–204.

Oswald, K. J., French, B.W., Nielson, C., Bagley, M. (2012) Assessment of fitness costs in Cry3Bb1-resistant and susceptible western corn rootworm (Coleoptera: Chrysomelidae) laboratory colonies. *Journal of Applied Entomology*, Volume 136,10: 730-740

Tabashnik, B. E., & Gould, F. (2012) Delaying corn rootworm resistance to Bt corn. *Journal of economic entomology*, 105(3), 767-776.

<http://www.ingentaconnect.com/content/esa/jee/2012/00000105/00000003/art00003>

(2) Risk mitigation We think the proposals made by EFSA are interesting, but will not work in practice. In the long run, this maize is not sufficiently toxic (high dosage) to effectively control the rootworm. Commercial cultivation of Maize 59122 might even exacerbate the problem by allowing the emergence of resistances in the pest insects and by enabling the selection of pest populations with an even higher fitness as seen in the case of MON88017 (Oswald et al., 2012).

Oswald, K. J., French, B.W., Nielson, C., Bagley, M. (2012) Assessment of fitness costs in Cry3Bb1-resistant and susceptible western corn rootworm (Coleoptera: Chrysomelidae) laboratory colonies. *Journal of Applied Entomology*, Volume 136,10: 730-740

(3) Monitoring The monitoring plan must be rejected since no case specific monitoring has been requested for most relevant non-target organisms or for possible health effects.

(4) more updated informations necessary The application for Maize 59122 was filed by Pioneer in 2007. Member States also had an option to comment on the application in 2007. There is no option for Member States to comment on more recent scientific studies that might change their stance on the product. There seems to be a systematic flaw in the approval process that should be revised.

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