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Institute for Independent
Impact Assessment in
Biotechnology

Genetically engineered maize 1507: Industry and EFSA disguise true content of Bt toxin in the plants

Data insufficient to conclude on the safety of the plants

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Content

Summary:

Testbiotech made the first meta-analysis of some of the data on genetically engineered maize 1507 submitted by industry to authorities in the EU, the US, Australia and New Zealand for their approval procedures. Correct data on the Bt content of the plants is a fundamental prerequisite for a reliable environmental risk assessment of these plants. However the assessment and comparison of the data has revealed huge variations in the amounts of Bt toxin in the plants, and further shows that 1507 maize plants are neither sufficiently homogenous nor predictable. On the contrary, they exhibit much greater differences in their quality than suggested by the European Food Safety Authority (EFSA). It is likely that breeding methods, regional environmental conditions, the application of herbicides, climate changes and other factors will substantially impact the Bt content of the plants. The data presented by industry so far do not allow any conclusion to be drawn on the actual Bt content in plants grown in specific environmentally stressful conditions. There is, in addition, hardly any essential data available on, for example, the Bt content in the roots. Despite all the uncertainties in assessing the Bt content in the plants, no further data from independent investigations has been requested and the true content of Bt toxin in the plants remains more or less unknown. Hence, permission for commercial cultivation in the EU based upon current data should not be given.

Introduction:

Genetically engineered maize 1507 which produces a Bt toxin (Cry1F) is close to authorisation in the EU. In December 2013, Testbiotech had already pointed out deficiencies in the environmental risk assessment of this maize (Then, 2013). Now Testbiotech has completed a comparison of the data on the content of Bt toxins in the plants submitted by industry to the European Food Safety Authority (Stauffer & Rivas, 1999; Stauffer, 2000; Pavely, 2002) with those data that were made available in the context of other EU applications (Philipps, 2008) and other regions (US EPA, 2010; Pioneer Hi-Bred International, 2001).

Results:

The comparison of the data shows that the differences in the content of the Bt toxin in the plants are much greater than assumed so far. In some cases the Bt content can vary more than tenfold (see Table 1), depending on breeding methods, plant growth, regional environmental conditions, application of herbicides and other factors or stressors. Any assessment of the data is further complicated by the units used by Pioneer / DuPont: The Bt content was measured in relation to the total extractable protein (TEP in pg/µg), which is difficult to transfer into the usual units which normally refer to dry weight of the plants.

Table 1: Overview of some maximum/ minimum data on the content of Bt toxin in maize 1507 taken from dossiers of industry (TEP, total extractable protein, pg/µg)

Tissue	Lowest concentration in specific plant tissue	mean	Highest concent. in specific plant tissue	mean	Source	Factor of variation between lowest and highest concentration
LEAF						
Leaf, Chile	56,6	110,9			Stauffer & Rivas, 1999	
Leaf, Italy and France, treated with glufosinate			651,4	348	Stauffer, 2000	11,5 fold
POLLEN						
Pollen, Chile	113,4	135,5			Stauffer & Rivas, 1999	
Pollen, Italy and France, treated with glufosinate			630,8	190,5	Stauffer, 2000	5,6 fold
SILK						
Silk Chile	26,8	50,3			Stauffer & Rivas, 1999	
Silk, Italy and France, treated with glufosinate			265,3	133	Staufer, 2000	10 fold
STALK						
Stalk, France, Bulgaria, Italy (Hybrid S)	211	395			Pavely, 2002	
Stalk USA (inbred)			2750	1770	Pioneer HiBred, 2001	13 fold

Tissue	Lowest concentration in specific plant tissue	mean	Highest concent. in specific plant tissue	mean	Source	Factor of variation between lowest and highest concentration
GRAIN						
Grain, France, Bulgaria, Italy (Hybrid S)	35	74			Pavely, 2002	
Grain USA, (inbred)			361	231	Pioneer HiBred, 2001	10 fold
FORAGE						
Whole plant forage, France, Bulgaria, Italy (Hybrid S)	0	396			Pavely, 2002	
Whole plant forage, USA (inbred)			1500	1110	Pioneer HiBred, 2001	3 fold (comparing mean) and more
WHOLE PLANT						
Whole plant (R1) Italy and France, treated with glufosinate	323,4	671,9			Stauffer, 2000	
Whole plant France, Bulgaria, Italy (R1)			2190	1310	Pavely, 2002	7 fold
SENESCENT PLANT						
Senescent plant, Italy and France, treated with glufosinate	171,2	198,9			Stauffer, 2000	
Senescence plant, Chile, inbred			968,3	677,5	Stauffer & Rivas, 1999	5,6 fold

Looking at the data it is evident that there is no consistent method of data acquisition or evaluation. For example, although the application of the herbicide glufosinate appears to have a substantial impact on the Bt content in the plants, only a very small amount of comparative data was submitted to the authorities. Furthermore, important data on, for example, the Bt content in the roots are largely missing.

It must also be pointed out that the methods used for measuring the Bt content in the plants were not validated by other laboratories and therefore cannot be considered to be sufficiently reliable. It is known that small differences in the protocols used to determine the Bt content can lead to huge differences in the results (Székács, et al., 2011).

Further, the methods used by industry appear to be substantially flawed; in several parts of the plants, researchers were unable to find PAT protein that confers resistance to glufosinate, even though it is obviously present.

The US authorities also mention some of the inconsistencies that Testbiotech found:

> „USDA bar Protein expression values indicated substantial variability in protein levels for Cry1F in the tissues sampled. No definitive conclusions could be reached from the data presented when comparing levels of Cry1F in hybrid 1507 and inbred 1507 when examining pollen, silk, stalk, leaf, grain, whole plant and senescent whole plant samples. Since these hybrids and inbreds were grown in areas of Chile with similar climatic extremes to the maize growing areas of the U.S., it is anticipated that these values will represent those to be expected in the U.S. cornbelt. PAT expression was also not readily distinguishable when comparing inbred and hybrid expression values. The inability to detect PAT protein in the majority of samples, except leaf, is somewhat puzzling in that the plants demonstrated clear glufosinate tolerance at all field sites. Given the generally strong, non-tissue specific expression levels typically associated with the CaMV 35S promoter (driving pat expression), it is not readily apparent why more PAT protein was not detected in more samples. Its presence in leaf tissue was expected, however, the reason for the absence in many of these samples is less than clear.“

Despite all the gaps in the data on Bt content in the plants as provided by industry, no further data from independent investigations has been requested by the authorities in the EU, the US or Australia/ New Zealand. Consequently, the true content of Bt toxin in the plants remains unknown.

A comparison with the opinion of EFSA

The European Food Safety Authority (EFSA) has taken very little notice of the question of variations in Bt content in the plants. EFSA (2005) states (using other units than Pioneer) that:

> "As additional information, the applicant submitted tables including recalculated the data from Cry1F ELISA experiments. The data are presented on a ng Cry1F protein/mg tissue dry weight basis and show that the expression values fall within the same order of magnitude for cultivation in different years and at different locations. Maximum expression (on a tissue dry

> weight basis) was found in pollen (average 20.0 and maximum 29.3 ng Cry1F protein/mg tissue dry weight). The values for whole plant extracts ranged between 1.0 and 6.9 ng Cry1F protein/mg tissue dry weight and for kernels between 1.2 to 3.1 ng Cry1F protein/mg tissue dry weight. The expression of Cry1F was not influenced by the application of glufosinate."

The average and maximum levels of Bt content in the pollen given by EFSA are clearly too low (see EFSA 2011). EFSA says that the range of variations in the Bt content in the grains is about threefold, while existing data show a range of ten. Further, data from Stauffer (2000) show that when glufosinate is applied to the plants it has a substantial impact on the range of Bt toxin content in the plants. For example, Stauffer (2000) examined the application of glufosinate by spraying and by painting the leaves which resulted in huge differences in the content of the Bt toxin. Thus, the opinion of EFSA (2005) is flawed.

In a footnote from 2012, even EFSA admits that the data as provided by industry are not sufficiently reliable, because meanwhile improved methods were available:

> „The EFSA GMO Panel used a more conservative value of 32 µg/g dw in the ERA of maize 1507 pollen (EFSA, 2011) based on US EPA data (US EPA, 2001, 2005) for which the applicant used an improved protein extraction and quantification system.“

Despite this statement, EFSA did not ask for new data from industry or independent investigations. Instead, reference was made to data from the US – but also these data seem to be clearly outdated. As the overview of existing data from the US shows (US EPA, 2010), the US authority only relies on data from Pavely (2002), which also were used by EFSA.

In the light of these findings, it is not astonishing that the Bt content in the pollen (which has finally been set at 32 ng/mg dry weight), is qualified by EFSA only as an estimation (EFSA 2011).

Conclusions:

Based on existing data it has to be assumed that the most crucial traits of genetically engineered 1507 maize plants that are about to be approved are neither sufficiently homogenous nor predictable enough. Breeding methods, regional environmental conditions, application of herbicides and other factors or stressors are all liable to affect the content of the Bt toxin which can presumably vary much more than shown by these data. To date there have been no systematic and independent investigations into influencing factors or the maximum possible content of the toxin in the plants. Therefore, the existing data are insufficient to draw conclusions on how high or low the toxin concentration would actually be in extreme cases where the plants are exposed to higher climatic stress. These huge uncertainties in risk assessment are for example relevant for protected butterfly species, pollinators, soil organisms, wild animals as well as farm animals.

In consequence, no conclusions can be reached on the safety of the plants. Because EFSA did not request reliable data on the Bt content in the plants, fundamental parts of the risk assessment are flawed, and the whole risk assessment collapses like a house of cards as soon as the details are analysed.

EU regulations request a high level of protection for human health and the environment and a reliable risk assessment by EFSA, therefore market approval for 1507 should therefore be withheld.

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