How much Bt toxin do genetically engineered MON810 maize plants actually produce?

Some results from Greenpeace field investigations

MON810 maize is genetically modified to produce a modified insecticide (Cry1Ab) that naturally occurs in the soil bacterium Bacillus thuringiensis (Bt). The production of this toxin is supposed to protect the maize plants from European corn borer (ECB, Ostrinia nubilalis) larvae.

Information is rarely published showing the real concentrations of Bt toxin in MON810 maize plants, and for this reason in 2006 Greenpeace took more than 600 samples from fields in Germany and Spain to be analysed in a special laboratory. The result was that there is a huge range of concentrations of the Bt toxin in the plants. Plants from the same field can vary by up to a factor of 100. The data from the Greenpeace report[1] give rise to new concerns related to the safety and product quality of genetically engineered (GE) maize.

An overview of the available scientific literature on Bt maize MON810 shows that the actual Bt concentration that is produced by MON810 plants in the field still remains largely unknown – even more than ten years since MON810 was grown commercially for the first time. Hardly any studies have addressed the impact of the environment on these GE plants, and there are virtually no data that show how the concentration of Bt toxin in the plants develops under real field conditions of commercial growing (such as different climatic or stress conditions). This lack of research gives the impression that Bt plants usually produce steady and consistent Bt levels, and are more or less independent from any environmental impact or specific genetic conditions[2].

Detailed data on the Bt production of MON810 plants were first published in April 2007 by Nguyen & Jehle[3], shortly before the Greenpeace report was finished. Their publication shows that there is a huge degree of variation between the individual plants, as well as significant differences between the field sites. Furthermore, the degree of Bt toxin concentration varies according to the season.

Like Nguyen and Jehle, Greenpeace took leaf samples in the 2006 growing season of commercially cultivated MON810 maize plants in Germany and Spain in order to determine the Bt toxin (Cry1Ab) concentration. In contrast to Jehle, samples were taken more often from different fields in order to obtain a clearer picture of the changes during the seasons. More than 600 samples from 12 different fields were analysed: between May and September/October 2006, plant samples were taken weekly on two fields in Bavaria, four fields in Brandenburg and bi-weekly on five fields in Spain. In addition, a Monsanto test field in North Rhine-Westphalia was sampled three times in three weeks in July/August 2006.

The Greenpeace investigation shows a surprising number of plants that contained only very low Bt toxin levels. However, high levels were also observed in some plants. The variation found across the same field on the same day was considerable, and can reach a factor of 100 times. This confirms in general the results of Nguyen & Jehle's 2007 study, which concluded that "the monitoring of Cry1Ab expression [of MON810 plants] showed that the Cry1Ab concentrations varied strongly between different plant individuals." Indeed, the actual variation found by Greenpeace was even larger than the one found by Nguyen and Jehle. Greenpeace also found a constant change in

[1]Greenpeace 2007. How much Bt toxin do genetically engineered MON810 maize plants actually produce? available at www.greenpeace.de .

[2]Monsanto 2002. Safety assessment of YieldGard insect-protected event MON810. Published by www.agbios.com as "Product Safety Description". http://agbios.com/docroot/decdocs/02-269-010.pdf

[3]Nguyen, H. T. & J. A. Jehle 2007. Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize MON810. *Journal of Plant Diseases and Protection* 114(2): 820-887.

the Bt toxin thanks to the growing season, with the highest point reached in the summer months of July and August.

In total, the Bt concentrations recorded certainly failed to match those available from Monsanto for cultivation approval in the US and the EU, with a mean of 9.4 µg Bt/ g fresh weight (fw) given for field sites in US, and mean averages from 8.6 to 12.2 µg/ g fw in Europe.[4]

The data from Greenpeace are more in accordance with the results of Nguyen & Jehle (2007), who also found lower Bt concentrations than the official Monsanto figures (2.4-6.4 µg Bt/g fw in the top leaf). The data recorded by Greenpeace however deviate still further from the data published so far. The statistical analyses shows a mean average concentration of about 0.5-2.2 µg Bt/g fw (in the top leaf), with a huge range of variability. In one case, an individual plant in one of the Bavarian fields was measured with a Bt concentration of 0.1 µg Bt/g fw, compared with another plant in the same field whose Bt concentration was 100 times higher (>10 µg Bt/g fw). On other dates, the highest Bt levels were 10-20 times higher than the lowest levels. Furthermore, in 8% of the leaf sample in the fields in Brandenburg, no Bt toxin could be detected at all.

Region	Number of samples	Median	Mean average	Max.	Min.	Number of samples with no detectable Bt
		[µg Bt/g FW]				
Bavaria	115	1.3	2.2	0.1	10.9	0
Brandenburg	193	0.7	1.3	0	13.0	28
Spain	129	0.6	1.6	0	14.8	7
Borken	136	0.4	0.5	0	3.4	1
Monsanto			9.4			
Nguyen & Jehle			2.4 - 6.4			1

Table: Comparison of mean average and median of Bt concentrations of MON810 leaves. If stated: top leaf, fresh weight.

[4]Monsanto 2002. Safety assessment of YieldGard insect-protected event MON810. Published by agbios.com as Product Safety Description. Available at http://agbios.com/docroot/decdocs/02-269-010.pdf

The presented results might be preliminary in some aspects but neverthelesse raise far-reaching questions about the safety and technical quality of the MON810 plants, as well as some fundamental methodological questions. Some of the most urgent conclusions from the Greenpeace report are as follows:

1. The variation of Bt concentrations

Since Bt concentrations in the field can vary greatly even between neighbouring plants, the MON810 plants do not appear to be stable in their biological traits. The high variation in Bt concentrations could potentially be attributed to genetic or environmental factors (e.g. weather or soil conditions), or to a combination of both. Nguyen & Jehle (2007) not only found high variation between plants in the same field, but also statistically significant differences between different locations in Germany. Since the reasons for such differences and the range of variation cannot be identified, the commercial cultivation of the crops should be stopped to avoid any interactions with the environment that could lead to adverse and unpredictable effects.

To investigate these questions further, studies should be conducted under contained conditions to study the environmental effects (e.g. drought, moisture, temperature, soil and nutrients) on the plants. To date, no such studies have been published.

2. The risk assessment of the plants

Risk assessments on non-target organisms or feeding studies in which the actual Bt concentrations have not been determined appear to be of little use. Studies in which the toxin concentration is unknown cannot be used to approve the commercial growing of these plants.

3. The actual Bt toxin concentrations

If the Bt toxin in GE Bt plants were more effective in considerably lower concentrations than previously described, then this would not be identical with the naturally occurring Bt toxin. This would annul a central aspect of the EU cultivation approval, which is based on the assumption that the Bt toxin in plants can in general be equated with the natural Bt protein from

soil bacteria (see also Hilbeck & Schmidt 2006[5]).

However, if the toxin is ineffective in such low concentrations as recorded above, then serious concerns need to be raised about the effectiveness of the plants to control ECB larvae. Additional problems would then also concern Insect Resistance Management, because sub-lethal toxin doses could accelerate resistance development.

4. The methods to determine Bt concentrations

The methods used by Monsanto to determine the Bt concentration of their original MON810 plants are not publicly available. In order to make a reliable comparison of new data with data from Monsanto, it is essential that the test protocols as well as the original data are published. All interested laboratories need unrestricted access to relevant sample material. The authorities need to define standardised and sufficiently reliable methods to determine Bt concentrations for risk assessment studies and for post-market monitoring.

Until the open questions regarding risk assessment, monitoring and product quality have been clarified, the commercial cultivation of MON810 needs to be stopped because the legal basis for putting MON810 onto the market has not been fulfilled.

Greenpeace demands:

- Stop the cultivation and authorisation of GE maize plants in the EU and elsewhere.
- Do not release genetically modified organisms into the environment.

[5]Hilbeck, A. and J. E. U. Schmidt 2006. Another View on Bt Proteins – How Specific Are They and What Else Might They Do? *Biopestic. Int.* 2 (1): 1-50.