TESTBIOTECH Background 10 - 1 - 2013

Testbiotech comment on EFSA Scientific Opinion on an application (EFSA-GMO-NL-2009-70) for the placing on the market of genetically modified drought tolerant maize MON 87460 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto

Testbiotech e. V.
Institute for Independent Impact Assessment in Biotechnology

Christoph Then & Andreas Bauer Panskus

Maize MON87460 is a genetically engineered, drought tolerant maize, which is used in the EU in food and feed, import and processing. It was made drought tolerant by introducing a bacterial protein (cold shock protein, CSPB) that is assumed to protect the plants against various stressful conditions.

Molecular data

(1) The plants contain a DNA sequence that confers resistance to antibiotics (npt II). Despite the fact that gene excision technology was used in this case (Cre/lox site-specific recombination system of bacteriophage origin), the DNA was not removed from the plants. However, EU Directive 2001/18 requires the phasing out of this outdated technology as it may have adverse effects. As comments from Member States show, there is controversial debate about the actual risks these DNA sequences. Moreover, any additional DNA inserted into the plants genome may cause disturbance in gene regulation, causing unintended effects. That is why any DNA sequence inserted require additional risk assessment. Consequently, such additional DNA sequences should be avoided especially if this is technically possible. In applying for market authorisation for this product without removing the DNA sequence from the plant's genome, Monsanto apparently decided to ignore current EU regulations. Any authorisation of this product would therefore only encourage a continuation of the practise of ignoring EU regulations.

Apparently also EFSA sees considerable amount of scientific uncertainty in regard to DNA transferral which is covered by words such as "likely" or likelihood – which appear at least eleven times on six pages (p.28 to 34). More uncertainty into this assessment is caused by recent publication (overlooked by EFSA) of Chen et al., 2012 which showed relevant DNA can be be distributed widely and integrated in microbes in aquatic systems. Therefore the assessment of EFSA will need further elaboration.

(2) How the DNA sequence conferring drought resistance actually works is not understood in detail. The plants produce an additional protein that is normally found in bacteria under stressful conditions such as cold shock. This new protein is continuously produced in all the plant's tissues throughout the period of vegetation. The presumption is that the bacterial protein can bind to RNA in the cell and stabilise the RNA which otherwise would be degraded under stressful conditions (Phadtare & Severinov 2010). According to current theory the stabilisation of the plants´

endogenous RNA enables the plant to continue protein production under stressful conditions. As a result, the plants are presumed to become tolerant to stressful conditions such as drought and their yield suppression therefore less severe than other plants grown under the same conditions (Castiglioni et al., 2008). According to the documents presented by Monsanto, this effect can be observed under some stressful conditions, but not in all cases.

The technology interferes with the delicate balance of production, processing and degradation of RNA. Within the multiplex tasks of RNA and its processing it is known that even very short pieces of produced RNA can show biological activity which can be transmitted from the plant to the consumer (Zhang et al., 2011). Accordingly, great caution should be applied in the risk assessment of these crops.

Since the CSPB is continuously produced in the plants (irrespective of stressful or non-stressful conditions) and the reaction between the plants´ RNA and the CSPB is not very specific, a wide range of unintended effects can occur in the plants. These effects might be dependent on specific environmental conditions. To assess unintended effects and associated risks it is important to understand the mode of action of these proteins. However, EFSA is of the opinion that investigation of these details is not necessary as long as unintended effects in the plants are not proven. As the Authority writes in reaction to concerns of the experts of Member States:

> "Extensive description of the underlying mechanisms was not considered needed by the EFSA GMO Panel given that phenotypic, agronomic and compositional analyses have not identified unintended effects."

Since this statement (which can be described as non-scientific and in contradiction to actual findings) must be interpreted as the basis underpinning EFSA´s risk assessment in this case, it necessarily gives rise to a high level of uncertainty. Methods such as screening the transkriptom and proteom were not applied, although these could help to identify relevant effects. Moreover, such methods are necessary to assess the true biological relevance of several additional open reading frames that were identified as a result of the process of genetic engineering.

Thus, based on current molecular data, no decision can be taken upon the safety of the plants. In awareness of (1) the many comments from Member States, which were not taken into account in their substance, of (2) the resistance to antibiotics and (3) considering that caution must be exercised in the case of technologies targeting the level of RNA production, the risk manager must proceed cautiously and reject the EFSA opinion.

References:

Castiglioni P., Warner D., Bensen R.J., Anstrom D.C., Harrison J., Stoecker M., Abad M., Kumar G., Salvador S., D'Ordine R., Navarro S., Back S., Fernandes M., Targolli J., Dasgupta S., Bonin C., Luethy M.H., Heard J.E. (2008) Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. Plant Physiology, 147, 446–455

Chen, J., Jin, M., Qiu, Z.G., Guo, C., Chen, Z.L., Shen Z.Q. Wang X.W., Li J.W. (2012) A Survey of Drug Resistance bla Genes Originating from Synthetic Plasmid Vectors in Six Chinese Rivers, Environ. Sci. Technol. 2012, 46, 13448−13454

Phadtare S. & Severinov, K. (2010) RNA remodeling and gene regulation by cold shock proteins, RNA Biology 7:6, 788-795

Zhang, L., Hou, D., Chen, X., Li, D., Zhu, L., Zhang, Y., Li, J., Bian, Z., Liang, X., Cai, X., Yin, Y., Wang, C., Zhang, T., Zhu, D., Zhang, D., Xu, J., Chen, Qu., Ba, Y., Liu, J., Wang, Q., Chen, J., Wang, J., Wang, M., Zhang, Q., Zhang, J., Zen, K., Zhang, C.Y. (2011) Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA, Cell Research: 1-10.

Comparative assessment (for compositional analysis and agronomic traits and phenotype)
The outcome of the field trials is open to various interpretations and fosters further uncertainties. Many significant differences were observed in composition data and phenotype (with and without environmental stress being applied) and are discussed controversially by the experts from Member States. Several experts pointed out that if differences were observed only in specific sites, these effects might depend on specific environmental conditions and should be investigated further. However, no further investigations were requested by EFSA.

EFSA concludes this debate by stating:

> Based on the results of a comparative analysis, the EFSA GMO Panel concludes that, besides the expression of the CspB and NPTII proteins, some differences were observed in the composition of forage and grain produced from maize MON 87460 compared with its conventional counterpart when grown under well-watered conditions. Given the magnitude of these changes and the characteristics of these endpoints, the EFSA GMO Panel concludes that the observed differences do not raise safety concern for humans or animals. The EFSA GMO Panel notes that under water-limited and other stressful conditions, maize MON 87460 can show enhanced agronomic performance characteristics and some differences in chemical composition in comparison with its conventional counterpart. Given the intended trait, the observed differences were not unexpected, and did not raise safety concerns. (highlighted by Testbiotech).

Thus, EFSA does not assume substantial equivalence in this case. But instead of requesting more detailed analysis (triggered by EFSA Guidance), the Authority is trying to avoid further discussion as long as the effects observed are not proven to pose specific risks. This cannot be taken as a scientifically sound risk assessment and “best possible scientific opinion” as required by Regulation 178/2002. Without looking into the details of the mechanisms, causes and potential effects of these differences, no conclusion can be drawn upon the safety of these plants.

To show that EFSA is not really interested in exploring the risks and true qualities of the plants, we only need to look at the outcome of the greenhouse experiments. Even though the plants were producing a cold shock protein, (according to the EFSA opinion) they did not show any advantage under cold stressful conditions in comparison with other maize plants. Instead of discussing these surprising finding, EFSA simply stated

> “Given the intended trait, the observed differences were not unexpected, and did indicate no safety concerns.”

In conclusion, EFSA´s risk assessment is flawed because of the severe limitations of current knowledge, high levels of uncertainties and questionable assumptions.

Toxicology
The feeding study with mice to examine the acute toxicity of the CSPB protein is not in line with OECD standards, nor in line with EFSA Guidance. For example, only 4.7 mg/kg was applied instead of a minimum of 2000 mg/kg as required by OECD. While EFSA suggests performing a repeated dose 28-day oral toxicity study, in this case there was only an acute oral toxicity test.

The outcome of this feeding study is a matter of scientific controversy, since some experts from Member States are concerned about changes in body weight, which were higher in comparison with the control group. In its answer to the request for further investigations, EFSA stated very vaguely:

> "The EFSA GMO Panel considered that acute toxicity testing of the newly expressed proteins is of little additional value for the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants."

To justify the assumption that no further toxicity tests are required, EFSA refers in its opinion to a history of safe use of the protein CSPB. *Bacillus subtilis* , which is a source for the CSPB is used in food production. However, we are not aware of a detailed analysis showing that the pattern of exposure will not be changed by the introduction of this maize into the food chain. Further, the protein as produced in the plants differs slightly from its native variants.

The fate of the protein under realistic conditions of ingestion and or processing of the plants was not examined. In this case, EFSA did not request any data on processing.

What is also missing is a ring-tested method that delivers reliable and comparable results in determining the true content of CSPB in the plants.

In conclusion, the risk assessment of EFSA on toxicity of the protein lacks scientific scrutiny and is driven by assumptions without sufficient justifications.

The maize was also tested in a 90 days feeding study which showed some effects such as reduction in the weight of some organs that were considered non-relevant by EFSA. The study suffers from the fact that the feed of the control group was contaminated with genetically engineered maize. Although not mentioned it is also likely that genetically engineered soybeans were part of the diet. As a result, specific effects caused by maize MON87460 might be masked.

No feeding studies were performed with maize stemming from various stressful conditions that are known to influence its composition. No long-term feeding studies, with inclusion of several generations, were conducted. Further, only particular usages in food and feed were considered, while the application is not restricted to such uses.

Consequently, the feeding study with the maize cannot be considered as sufficient to show the safety of the product.

Allergenicity
The comparison with known allergens shows similarities with a protein that is suspected to have allergenic potential, but no specific tests with serum samples were conducted. Further, no tests were performed to investigate adjuvant effects that can enhance immune reaction to known endogenous maize allergens. Specific risks for individuals with impaired immune function, for the elderly or infants (in comparison with the general population) known to carry a higher risk were not considered.

The tests used to demonstrate digestibility of isolated proteins are known to be unreliable under

realistic conditions if the protein is ingested with many other components.

As a result, allergenicity risk assessment is not conclusive.

Nutritional Assessment
There are no data on any processed food and feed.

The feeding study as performed with poultry showed some significant differences that require more investigation. Further, the feed for the control group was contaminated with genetically engineered plants - this is not in accordance with good laboratory practise.

The study did not include grain from MON 87460 plants produced under various stressful conditions known to influence its composition.

Environmental risk assessment
Testbiotech agrees with the comments of several Member States that much more data on spillage, persistence and invasiveness are needed before any decision can be taken on risks for the environment.

Others
As a recent legal dossier compiled by Professor Ludwig Kraemer shows, the decision not to monitor Effects on health at the stage of consumption of genetically engineered food, violates the requirements of EU regulations. Directive 2001/18 and Regulation 1829/2003 both require that potential adverse effects on human health of genetically modified plants are controlled during the use and consumption stage, including those cases where such effects are unlikely to occur. Thus, the EFSA opinion that monitoring of health effects is unnecessary, is wrong and contradicts current EU regulations.

References:
Kraemer, L. (2012) The consumption of genetically modified plants and the potential presence of herbicide residues, legal dossier compiled on behalf of Testbiotech, http://www.testbiotech.de/sites/default/files/Legal_Dossier_Kraemer_Pesticide_RA_PMP.pdf

Conclusion and recommendations:
The opinion of EFSA does not identify the true range of uncertainties (as requested by Regulation 178/2002) and the current limits of knowledge. The risk manager should therefore reject this opinion.

As for the risk manager’s concerns about socio-economic effects, it should be taken into account that this may render some advantages for farmers under drought conditions. However, there are other maize varieties available, derived from conventional breeding that are adapted to drought. There is no identifiable reason why this specific maize should be imported or cultivated.