Testbiotech Data Factsheet:
Insect- killing Soy MON87701 (Monsanto)

January 2012

Plant:
Soybean

Event name:
MON87701

Applicant:
Monsanto

Trait:
Insect resistance

Bt Toxin:
Cry1Ac

Transformation method:
Agrobacterium

Scope of application:
Food and feed, import and processing

Impact on European market:
Millions of tons of genetically engineered soybeans are imported into the European market. Most of it is used in animal feed.

General Information:
Insect-killing Soy MON87701 is engineered to produce insecticidal protein Cry1Ac. This is a Bt toxin which is known to enhance immune reactions (Vázquez-Padrón et al., 1999 and 2000). Soy is one of the most potent allergenic food plants, consequently, from a precautionary perspective, this protein should be avoided in these plants.

Cry1Ac is also a Bt toxin known for its synergistic effects with other Bt toxins (Sharma et al., 2010). Synergistic effects can become highly problematic for non-target organisms. Interactivity of the toxins or the toxins in combination with environmental toxins, bacteria, plant enzymes or pesticides can cause higher than expected toxicity and lower selectivity (Then, 2010). These effects can impact human health as well as ecosystems. The plants will go into food and feed and might, therefore, be mixed with other genetically engineered plants. Tests need to be carried out to determine potential accumulative or combinatorial effects.

Further, soybeans produce large amounts of protease inhibitors (trypsin inhibitors) that 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 Cry toxins. The extent to which the trypsin inhibitors will be destroyed by heat processing depends on the method used.

In general, the mode of action of Bt toxins is not fully understood. It 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. More recent research (Soberon et al., 2009) shows that there are mechanisms that might cause toxicity in other species and even in mammals. 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. Thus, risks for human health cannot be excluded by assumptions or considerations but only by empirical testing before market authorisation.

This soy exemplifies the unintended impact of the transgene on the plant metabolism: The level of Vitamin E is enhanced unintentionally in this soy when compared to the level in the control plants. But despite these findings, EFSA 2011, states that *"No indication was found in the molecular analysis and in the comparative compositional, phenotypic and agronomic analysis that the genetic modification of soybean MON 87701 resulted in any unintended changes."* This statement simply is wrong. These genetically engineered plants can not be regarded as being "substantially equivalent". Even according to Guidance of EFSA (2011c) MON87701 requires a "comprehensive risk assessment" and not only a "comparative risk assessment".

Feeding studies were repeated because they revealed significant effects in rats. The effects were not reproduced in the second feeding trial, but there were other significant effects. Further, higher mortality and other significant findings were shown in feeding trials with poultry fed with the genetically engineered soy. Despite these findings, no long-term and more detailed studies were conducted. Potential risks for human health are supported in a report by Gallagher (2010) dealing with kidney problems and immune reactions observed in feeding studies with genetically engineered eggplant, which also express a modified Cry1Ac protein.

All in all, this product has a wide range of risks and a high level of uncertainty concerning its safety. The risks might depend on the way the soybeans are processed (for example as sprouts or as heat processed feed) because this can impact the level of the Bt toxin, of allergenic active proteins and trypsin inhibitors. However, the effects of the different methods used for processing were not assessed by EFSA.

Specific risks and unintended effects

- Open reading frames were identified that can give rise to unintended gene products in the plants.
- According to experts from member states, the content of the additional proteins produced in the plant are highly variable (EFSA 2011b). 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 the Bt content in the plants (Then& Lorch, 2008).
- In comparison with its conventional counterparts, several significant differences in compositional analysis were observed. Most of the differences were not consistent over all field trials. The reason for this might be that these differences only emerge under particular environmental conditions. Concerning the level of Vitamin E, there were consistent findings for significant differences in all field trials. Thus these genetically engineered plants can not be regarded as being "substantially equivalent". According to Guidance of EFSA (2011c) these plants would require a "comprehensive risk assessment".
- In agronomic parameters, several significant differences were identified in comparison to the control plants. Most differences were not consistent over all field trials. The reason for

this might be that these differences only emerge under particular environmental conditions.

- Sharma et al. (2010) found synergistic effects of Cry1Ab and Cry1Ac in target pest insects. Further synergistic effects between Cry1Ac and other Bt toxins such as Cry2Ab2 and Cry1F are discussed in and Lee et al. (1996), Chakrabarti et al (1998) and Khasdan et al (2007). Synergistic interactivity between Cry2Ab2 and Cry1Ac has also been discussed in Stewart et al. (2001). Synergistic effects can become highly problematic for non target organisms: Interaction of the toxins with each other or with other compounds can cause higher toxicity and lower selectivity (Then, 2010). These effects may impact human and animal health as well as the protection of the ecosystems.
- Soybeans are known to cause severe allergic reactions. The newly introduced gene protein Cry1Ac is known to enhance immune reaction and might also enhance an immune response to the endogenous plant protein(s). Tests were performed on blood samples from individuals with a known allergy to soy proteins. Some of them showed a different reaction to the genetically engineered soybeans. This can signal altered allergic potential of the soybeans.
- Soybeans are known to produce compounds with hormonal activity. The content of these compounds might be changed by interference with the newly introduced gene constructs.
- These plants will be fed and might be eaten by mixing them with other genetically engineered plants. Tests have to be performed on potential accumulated effects such as combinatorial or accumulated effects.
- Some plant enzymes that diminish the digestion of proteins (protease inhibitors, for example, trypsin 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 soy produces large amounts of such inhibitors.
- Feeding studies revealed significant effects in rats and were repeated for this reason. The effects were not reproduced in the second feeding trial, but there were other significant effects. Further, there was higher mortality and other significant findings for poultry fed with genetically engineered soy. Potential risks for human and animal health are supported in a report by Gallagher (2010) dealing with kidney problems and immune reactions that were observed in feeding studies with genetically engineered eggplant which also express a modified Cry1Ac protein.

Type of feeding trial conducted:

- An acute toxicity study was performed, feeding isolated Cry1Ac proteins. These proteins were not isolated from the plants but produced by bacteria.
- Two feeding studies with the heated and processed material from the plants were performed to assess health effects, but none with sprouts or other raw material.
- A feeding study with poultry was performed to assess nutritional effects.

Overview of some shortcomings of EFSA opinion:

- Since these soybeans cannot be regarded as being substantially equivalent, EFSA´s guidance requires a comprehensive risk assessment (EFSA 2011c). This risk assessment described by EFSA as an alternative to its standard comparative risk assessment, has neither been defined by EFSA nor was it applied in this case.
- No systematic investigation under various defined environmental conditions was conducted to determine interactions between the genome and the environment.
- There was no systematic investigation of changes in composition and agronomic performance under various defined environmental conditions.
- Functional stability of the transgene under various defined environmental conditions was not

shown. Genetic stability was only considered in the context of the hereditary of the gene constructs to following generations.

- In addition to the content of Vitamin E, several significant differences in the compositional analysis were found in comparison with its conventional counterparts. The differences were not consistent over all field trials. References were made to unspecific and questionable 'historical' data from industry unrelated to the actual field trials, e.g. the ILSI database. Since it is not sufficiently clear under which specific conditions these additional historical data were generated, this kind of comparison inevitably contains major uncertainties.
- Significant differences in agronomic performances should have been investigated in relation to interactions between the genome and the environment under defined environmental conditions.
- The feeding studies in rats and poultry showed a large number of significant findings that should have been investigated in much more detail.
- The significant findings in blood samples from individuals with a known allergy to soybeans, should have triggered more investigations with a much larger number of blood samples. Instead EFSA (2011b) stated in response to concerns of member states: *"The EFSA GMO Panel requested the applicant to comment on the observed differences (…) between the (…) MON 87701 and the control, in particular, when more spots can be seen with MON 87701 (…) and to identify (…) the spots corresponding to the known major soybean allergens. The applicant gave general comments that did not raise concern."*
- No investigations were conducted to assess the impact of a permanent ingestion of these plants on the intestinal microbial composition in human and animals.
- No assessment of combinatorial effects with other genetically engineered plants used in food and feed.
- There have been no feeding studies over the whole lifetime of animals and none including following generations.
- No endocrinological studies were performed to investigate potential impacts on the reproductive system
- No investigation conducted for DNA traces in animal tissue after feeding.
- No tests were performed to determine potential combinatorial or accumulated effects of the toxins nor of any other factors as other toxic compounds, bacteria, plant enzymes (trypsin inhibitors) and pesticides in mammals, despite the fact that Cry1Ac is known for potential synergies with other Bt toxins.
- No empirical investigation of the actual persistence of the Bt toxins and their potential accumulation in the environment.

Surveillance – Monitoring

- No plan for surveillance was made available that would allow identification of particular health impacts that might be related to the use of these genetically engineered plants in food and feed.
- The protocols used for conducting the measurements of the Bt toxins have not been fully published or evaluated by independent laboratories. As a result, independent institutions can hardly monitor the actual content of Bt concentration in the plants during cultivation or in food and feed products.

Documents and publications:

EFSA , 2011a, Panel on Genetically Modified Organisms (GMO); Scientific Opinion on application

(EFSA- GMO-BE-2010-79) for the placing on the market of insect resistant genetically modified soybean MON 87701 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal (2011); 9(7):2309 [31 pp.] doi:10.2903/j.efsa.2011.2309. Available online: www.efsa.europa.eu/efsajournal

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and selectivity. Environ Sci Pollut Res Int; 17(3):791-7

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