Testbiotech Data Factsheet: Herbicide Cocktail Soybean 356043 (Dupont/Pioneer Hi-Bred, brand *Optimum GAT Soybean*)

January 2012

Plant
Soybean

Event name:
DP356043

Applicant:
DuPont/ Pioneer Hi-Bred International Inc.

Trait:
herbicide tolerance

Herbicide:
glyphosate (brandnames such as Roundup or Touchdown)
ALS inhibitors

Transformation method:
Particle bombardment

Scope of application:
Food, feed, import, processing

Impact on European market:
The impact on the European market might be considerable if approved. Millions of tons of genetically engineered soybeans are imported into the European market. Most of it is used in animal feed.

General information:
The application for soybean 356043 is connected to the emergence of glyphosate resistant weeds in countries where glyphosate resistant crops (so-called Roundup Ready crops) dominate the agricultural landscape. Since in many regions these crops (such as soybeans) are cultivated on large scale, the weed adopted to the spraying of glyphosate and there is an massive increase in usage of the herbizide (Benbrook 2009; Grube 2011). In July 2011, a scientific database listed 21 different glyphosate resistant weeds (http://www.weedscience.org). Industry is therefore trying to develop transgenic crops with tolerances to other pesticides (such as glufosinate, ALS-inhibitors or 2,4-D) as a possible solution.

Soybean 356043 was one of the first genetically engineered crops with resistance to ALS-inhibitors to be approved for cultivation. Transgenic crops that tolerate ALS-inhibitors are equally as problematic as glyphosate in terms of pesticide resistance. In July 2011, 109 weeds worldwide were considered resistant to ALS-inhibitors, many of them in soybean fields (http://www.weedscience.org).

Soybean 356043 can not be seen as being "substantial equivalent", CERA database (http://cera-gmc.org), lists it as the first crop being filed for market application, not fulfilling this criterion. EFSA lists level of fatty acids and amino acids being changed unintentionally by genetic engineering (EFSA 2011a):
„In the composition, differences were identified between 356043 soybean and its conventional counterpart in the newly expressed proteins Glycine max-HRA and GAT4601, and the levels of the fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid and the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG).“ Thus even according to Guidance of EFSA (2011c) a "comprehensive risk assessment" and not only a "comparative risk assessment" would have been necessary.

The higher level of the amino acids are caused by the glyphosate-N-acetyltransferase gene sequence (gat4601) that is used for achieving glyphosate tolerance. The gat gene produces two aminoacids as by-products (N-acetyl-aspartate, NAA and N-acetyl-glutamate, NAG). They are present in soybean 356043 at 230 times the levels found in conventional soybean (Pioneer, 2006). Acetyl-aspartate is usually found in the mammalian nervous system and is neurotoxic in high doses. Toxicity tests that were conducted with acetyl-aspartate and acetyl-glutamate and soybean 356043 showed numerous significant findings.

Further, the gat-gene produces new metabolites from glyphosate that are not present in other glyphosate tolerant soybeans such as the Roundup Ready soybean (in these plants the tolerance against glyphosate is based on the introduction of the epsps gene sequence). As EFSA described it in a previous opinion (EFSA 2009):
„Metabolism studies in genetically modified soybeans and maize containing the glyphosate-N-acetyltransferase (GAT) gene demonstrated that new metabolites are formed which were not observed in conventional crops or in glyphosate tolerant crops containing the modified 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) gene. The major metabolite in the new maize and soybean varieties under consideration is N-acetyl-glyphosate. Parent glyphosate, N-acetyl-aminomethyl phosphonic acid (N-acetyl-AMPA) and aminomethyl phosphonicacid (AMPA) were found in low concentrations in the edible parts of the crops.“

AMPA and N-acetyl-AMPA are both considered to have toxicity similar to that of glyphosate. This leads to a combination of potentially hazardous residues from spraying. As recent overviews of scientific literature show (Antoniou, et al., 2010; Benachour, et al., 2007; Paganelli et al., 2010; PAN AP 2009; Then 2011), the toxicity of glyphosate, its metabolites and its additive like POEA (polyoxyethylene alkylamine) need to be re-evaluated. The additive POEA is even more toxic than glyphosate in the plants. The toxicity of glyphosate currently is under revision by the EU with its result being expected in 2012 but meanwhile likely to be delayed (see EU Commission, 2002; Antoniou et al., 2011).

Specific risks and unintended effects

- The plants contain novel synthetic genes as well as novel synthetic promoters.
- Compositional analysis showed several significant differences as compared to their conventional counterparts.
- Soybean 356043 is not substantially equivalent to conventional soy: thus comprehensive risk assessment has to be conducted.
- Soybean 356043 produces high quantities of amino acids N-Acetylaspartate and N-Acetylglutamate.
- In agronomic parameters, several significant differences were identified in comparison to the control plants. When analysed by site, statistically significant differences for seedling vigour and plant height were observed in several trials. The differences were not consistent

over all field trials. The reason for this might be that these differences only emerge under particular environmental conditions. Several investigations show that genetically engineered plants can exhibit unexpected reactions under stress conditions (see for example: Matthews et al., 2005).

- A 90 day feeding study with rats showed significant differences compared to the control group in some blood parameters.
- A 42 day feeding study with chickens showed higher liver weight in males fed meal from soybean 356043 treated with the target herbicides.
- Repeated dose studies with purified GAT and HRA proteins showed numerous significant findings.
- Soybeans are known to cause severe allergic reactions. The newly introduced gene construct might, for example, enhance an immune response to these endogenous plant proteins.
- 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 go into feed and might be mixed with other genetically engineered plants. Tests need to be carried out to determine potential accumulative or combinatorial effects.
- Plants contain residues from spraying with herbicide formulations and their metabolites such as AMPA and N-acetyl AMPA , that has a similar toxicity to glyphosate.
- The plants are made tolerant to glyphosate preparations by introducing a gene construct for the EPSPS enzyme. As recent overviews of scientific literature show (Antoniou, et al., 2010; Benachour, et al., 2007; Paganelli et al., 2010; PAN AP 2009; Then 2011), the toxicity of glyphosate, its metabolites and its additives such as POEA (polyoxyethylene alkylamine) need to be re-evaluated.

Type of feeding trial conducted:

- acute oral toxicity study in mice (E. coli-produced GAT4601 and E. Coli produced GM-HRA proteins)
- 28 day repeated-dose toxicity study with E. coli-produced GAT4601 and E. Coli produced GM-HRA proteins in rats
- Two generation study with E. coli-produced GAT4601 and E. Coli produced GM-HRA proteins in rats
- 42 day nutritional study in poultry with the soybean
- 90 day subchronic study in rats with the soybean

Shortcomings of EFSA opinion:

- Since these soybeans cannot be regarded as being substantially equivalent, EFSA´s guidance requires comprehensive risk assessment (EFSA 2011c). This risk assessment, which is described by EFSA as an alternative to its standard comparative risk assessment, has neither been defined by EFSA nor was it explicitly applied in this case.
- Field trials were only conducted during one cultivation period.
- No investigations were conducted to determine changes in plant gene activity or metabolic profile under various defined environmental conditions.
- No investigation under defined environmental conditions was conducted to determine interactions between the genome and the environment.
- There was no detailed investigation of changes in composition and agronomic performance under various defined environmental conditions.
- Significant differences found in some of the field trials were dismissed without considering specific interactions between the genome and the environment.

- Significant differences in agronomic performances should have been investigated in relation to interactions between the genome and the environment under defined environmental conditions.
- Risks were not investigated in detail despite significant findings from feeding trials indicating potential negative impacts on human and animal health.
- there have been no feeding studies with the plants over the whole lifetime of animals and none including following generations.
- The proteins used for acute and repeated dose toxicity tests were produced by bacteria. The toxicity maybe different, if plant material is being used.
- 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 investigation was conducted for DNA traces in animal tissue after feeding.
- No assessment of combinatorial effects with other genetically engineered plants used in food and feed was conducted.
- No endocrinological studies were performed to investigate potential impacts on the reproductive system, despite the fact that soy is producing hormonal active substances that might have been changed unintentionally.
- No assessment of risks stemming from residues from spraying with the pesticide formulations and their metabolites was conducted.

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.
- Monitoring of health effects has to include the risks associated with the spraying of glyphosate formulations and their residues in the plants.

Documents und publications:

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EFSA (2011b) Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 9(5): 2150, doi:10.2903/j.efsa.2011.2150.

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Paganelli, A., Gnazzo, V., Acosta, H., López, S. L., Carrasco, A. E. (2010) Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signalling. Chem. Res. Toxicol., August 9. pubs.acs.org/doi/abs/10.1021/tx1001749

PAN AP, Pesticide Action Network Asian Pacific (2009) Monograph on Glyphosate,
http://www.panap.net/en/p/post/pesticides-info-database/115

Then, C., 2011, Vorsicht „Giftmischer“: Gentechnisch veränderte Pflanzen in Futter-und Lebensmitteln, ein Testbiotech-Report,
http://www.testbiotech.de/sites/default/files/Testbiotech_Giftmischer_April_2011.pdf

Links:
The International Survey of Herbicide Resistant Weeds.
ALS inhibitors (B/2) resistant weeds by species and country:
http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=3&FmHRACGroup=Go

The International Survey of Herbicide Resistant Weeds.
Glycines (G/9) resistant weeds by species and country:
http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=12&FmHRACGroup=Go