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Testbiotech comment on the Scientific opinion on an application by Dow AgroSciences LLC (EFSA-GMO-NL-2012-106) for the placing on the market of genetically modified herbicide-tolerant soybean DAS-44406-6 for food and feed uses, import and processing under Regulation (EC) No 1829/2003

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Introduction

Soybean DAS-44406-6 produced by Dow AgroSciences expresses three proteins (EFSA 2017a):

- 2mEPSPS conferring tolerance to glyphosate-based herbicides,
- AAD-12 conferring tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and other related phenoxy herbicides and
- PAT protein conferring tolerance to glufosinate ammonium-based herbicides.

This genetically engineered soybean was created to combat problems arising from an increasing number of herbicide resistant weeds in countries where genetically engineered plants are cultivated.

Molecular characterisation

The expression of the additional enzymes was only measured under field conditions in the US. It is unclear to which extent specific environmental conditions can influence the overall concentration of the toxins in the plants. The plants should have been subjected to a much broader range of environmental conditions to obtain reliable data on gene expression and functional genetic stability. Environmental stress can also cause unexpected patterns of expression in the newly introduced DNA (see Trtikova et al., 2015).

Further, all parts of the plants should be taken into account for risk assessment. Expression data have to be considered as one of the starting points in the risk assessment of the plants, so the assessment of the data cannot be reduced to those parts of the plants entering the food chain.

Comparative analysis (for compositional analysis and agronomic traits and the phenotype)

Field trials for the comparative and agronomic assessment of soybean DAS-44406-6 were carried out in the United States at 10 sites in 2010 and at one site in 2012. No field trials were conducted in other soybean producing regions such as Argentina and Brazil.

Regarding agronomic traits, significant differences were found in pod count, seed count and (reduced) yield.

Regarding composition, following differences were found:

• DAS-44406-6/untreated
27 significantly different endpoints, five endpoints under equivalence category III or IV.

• DAS-44406-6/2,4-D
25 significantly different endpoints, four endpoints under equivalence category III or IV.

• DAS-44406-6/glyphosate
22 significantly different endpoints, two endpoints under equivalence category III or IV.

• DAS-44406-6/glufosinate
22 significantly different endpoints, three endpoints under equivalence category III or IV.

• DAS-44406-6/2,4-D+glyphosate+glufosinate
23 significantly different endpoints, two endpoints under equivalence category III or IV

Significant differences were found in:

- NDF, total dietary fiber, ash, carbohydrates, protein and moisture,
- copper copper, potassium, selenium and zinc,
- aspartic acid, cystine, isoleucine, leucine, lysine, tryptophan and tyrosine, tryptophan and tyrosine,
- palmitic (16:0), oleic (18:1), linoleic (18:2), and linolenic (18:3), arachidic (20:0), eicosenoic (20:1) and behenic (20:0) fatty acids,
- thiamine, riboflavin, folic acid, ascorbic acid, alpha tocopherol, gamma tocopherol and total tocopherol,
- raffinose, total daidzein equivalents and total genistein equivalents.

EFSA´s own guidance states that non-equivalence is more likely than equivalence for all significant findings that fall under equivalence category III or IV. Therefore, the genetically engineered soybean has to be considered to be different from its isogenic comparator in regard to several compounds. These findings are relevant and deserve more detailed investigations regardless of whether or not the specific compound raises safety concerns.

Given this wide range of biologically relevant differences, it is not acceptable that EFSA failed to require further studies e.g.

- Omics studies (proteomics, transcriptomics, metabolomics) to assist the compositional analysis and the assessment of the phenotypical changes.
- Investigations of changes in content of miRNA which can be taken up at from the gut and render biological effects across border of life domains. It should be noted that EFSA´s answer to questions from experts from Member States (EFSA 2017b) are not in line with latest findings on uptake and biological effects of miRNA. There are several more recent studies supporting the findings of Zhang et al (2012).
- Exposing the plants to a wide range of defined biotic or abiotic stressors to assess the true range of possible changes in the plants´ composition
- Including more varieties inheriting the trait to investigate how the gene constructs interact with the genetic background of the plants.

- Subchronic and chronic feeding trials with the whole plants to assess potential health effects. It should be noted that the 90-day feeding study provided by the company should have been rejected due to methodological weaknesses (see below).

EFSA does not address the fact that the overall number of significant results indicates the occurrence of unintended effects due to genetic modification, which would require more in depth investigation.

Further, effects on the immune system were completely ignored in the assessment of potential health impacts due to increased levels of lectins.

Based on the available data, no final conclusions can be drawn on the safety of the plants.

Toxicology

The applicant conducted acute toxicity studies, a feeding study on chickens, as well as three 28-day studies to confirm the safety of soybean DAS-44406-6. Further, a subchronic 90-day study was conducted. Two of the three 28-day studies were rejected by EFSA due to methodological flaws.

In the third 28-day study on mice, changes in blood parameters and other significant changes were found. Only a small number of animals were examined.

The 90-day study revealed several significant findings which were sex-related:

- Total white blood cell (WBC) and reticulocyte counts were slightly lower and statistically identified in males given soybean DAS-44406-6 as compared to those in the isoline non-transgenic controls.
- With females, soybean event DAS-44406-6 produced a statistically significant higher mean red blood cell (RBC) count, haemoglobin concentration and haematocrits compared to those in the isoline non-transgenic controls.

EFSA considered these findings to be “not toxicologically relevant.”

The study displays various weaknesses. For example, only one dose level was tested. Therefore, an assessment in regard to a possible dose-dependency of effects is not possible on the basis of a single test group.

Furthermore, according to EFSA, the study is not in line with the EFSA guidelines (EFSA, 2011):

- Functional testing was not performed;
- Animals were not housed individually;
- Only one dose level was used in the study;
- Power analysis was not performed.

It is particularly relevant that the soybeans used in the diet were not sprayed with the complementary herbicides.

Implementation Regulation (503/2013) requests a 90-day subchronic study as part of the risk assessment for all applications filed later than 2014. Taking this regulation into consideration, it is obvious that a full 90-day feeding study should have been requested since many biologically relevant differences were found between the event and its comparator, including compounds such as lectins. Further, soybeans sprayed with the complementary herbicides should have been included in the diet.

In addition, multigenerational studies should have been performed to assess the impact on the reproductive system.

In general, EFSA risk assessment suffers from the fact that residues from spraying with the complementary herbicide are considered to be outside the remit of the GMO panel. However, conclusions cannot be drawn on the safety of the imported products without detailed assessment of these residues. Due to the specific agricultural practices that go along with the cultivation of these herbicide resistant plants, there are, for example, specific patterns of applications, exposure, occurrence of specific metabolites and emergence of combinatorial effects that require special attention. Herbicide-resistant plants are meant to survive the application of the complementary herbicide while most other plants will die after short time. Thus, for example, residues of glufosinate and 2,4-D, its metabolites and additives to the formulated product might accumulate and interact in the plants. As the publication by Kleter et al. (2011) shows, using herbicides to spray genetically engineered herbicide-resistant plants does indeed lead to patterns of residues and exposure that need to be assessed in detail.

While it is true that Pesticide Regulations 396/2005 and 1107/2009 are relevant in this context, in practice, they are not sufficient to generate the data needed to assess the residues from spraying with complementary herbicides. Furthermore, according to a reasoned legal opinion drawn up by Kraemer (2012), residues from spraying with complementary herbicides do indeed have to be taken into account in the risk assessment of genetically engineered plants from a regulatory point of view.

There is a clear gap in the safety assessment of the genetically engineered soybeans that cannot be filled by adjustments to the MRLs applicable under the Pesticide Regulation. Consequently, the impact of residues from spraying must be assessed before the soybeans can be declared safe. The failure to do so poses real safety risks to humans, animals and the environment generally.

In conclusion, GMO risk assessment cannot be allowed to avoid its obligation to make sure that the applicant provides all the data needed to assess all derivative soybean products in all relevant health aspects.

There are good reasons for carrying out detailed assessments of the residues from spraying with the complementary herbicides:

- From scientific literature (not acknowledged by EFSA) it is known that metabolisation in crops tolerant to 2,4-D may lead to the production of the compound 2,4-DCP (2,4-Dichlorophenol). According to a review by Lurquin (2016), 2,4-DCP may cause negative metabolic and genotoxic effects, and, like 2,4-D, is listed as “a possible carcinogen based on inadequate evidence in humans and limited evidence in experimental animals” by IARC.
- A new study has recently linked 2,4-D with Non-Hodgkin Lymphoma (Smith et al., 2017).
- Some of the complementary herbicides for use on the DAS-44406-6 soybean will be phased out in Europe e.g. fluazifop and diclofop-Methyl.
- Glufosinate is suspected of having negative impacts on health (EFSA, 2005) and was already about to be phased out in the EU (EU Pesticides Database, 2017) because of being classified as showing reproductive toxicity.[1] Meanwhile their approval periods were exten-

[1] http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN

ded.[2] It is also known to cause residues from spraying if used as a complementary herbicide on genetically engineered plants.
- Combinatorial effects are likely to arise from interaction of residues from the mixed spraying of e.g. glyphosate, glufosinate and 2,4-D.
- Endocrine effects were found when young rats were exposed to soy milk in combination with glyphosate (Nardi et al, 2016).

In any case, both the EU pesticide regulation and the GMO regulation require a high level of protection for health and the environment. Thus, in regard to herbicide-resistant plants, specific assessment of residues from spraying with complementary herbicides must be considered to be a prerequisite for granting authorisation. In addition, cumulative effects have to be investigated if a plant contains or produces other compounds of potential toxicity.

It should be acknowledged, that no new methodology is needed to assess the health risks emerging from the combinatorial application of the herbicides and their potential interaction with the other plant constituents. There is, for example, no need to apply methods such as the Monte Carlo Risk Assessment (MCRA) because the majority of potential stressors can be expected to occur in a fixed combination and follow a specific pattern of exposure. Rather, the methods currently available (*in vivo* and / or *in vitro*) are sufficient to assess the health effects: For example, Regulation (EC) No 1907/2006 (REACH) provides guidance on how substances that are in fact mixtures (isomeric mixtures, MCS (multi-constituent substance) and UVCB (substances of unknown or variable composition, complex reaction products or biological materials) should be assessed for their PBT/vPvB (persistent, bioaccumulative and toxic) properties. In general, due to the nature of "substances of unknown or variable composition, complex reaction products or biological materials" it is not possible to make reliable predictions about the additive, or synergistic, or antagonistic mode of effects. Therefore, such substances have to be tested as a mixture, not as single compounds. For example, chronic feeding studies are a well-established method to generate the relevant data.

As a result, the toxicological assessment carried out by EFSA is not acceptable.

Allergenicity

There are several relevant issues regarding allergenicity and the immune system that were left aside in EFSA risk assessment:

- No non-IGE-mediated immune reactions were assessed although these effects must be considered relevant (Mills et al., 2013). This is especially relevant in this case since higher levels of lectins are present in comparison with the isogenic plants.
- The assessment did not take into account the risk for more vulnerable groups of the population such as infants (EFSA, 2010).
- The number of blood samples from patients with a known allergenicity to soybeans is much too small (10 samples) to draw any conclusions.
- An analysis published by EFSA experts and other scientists recently found that, in general, open questions remain regarding the allergenicity assessment of genetically engineered plants, especially in the case of engineered soybeans (Selb et al., 2017).

Overall, the assessment is insufficient to exclude impacts on the immune system.

[2]COMMISSION IMPLEMENTING REGULATION (EU) 2015/404, Official Journal of the European Union L 67/6, http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv%3AOJ.L_.2015.067.01.0006.01.ENG

Others
Monitoring should be case specific. Exact data on the exposure to the soybean should be made available. Possible health impacts must be monitored in detail. Controls regarding residues from spraying with glyphosate, glufosinate and 2,4-D have to be established. Accumulated effects that might stem from mixtures with other genetically engineered plants have to be taken into account in the monitoring plan.

Conclusions and recommendations
The risk assessment by EFSA is not acceptable in its present form. It does not identify knowledge gaps and uncertainties and fails to assess toxicity, impact on immune system and the reproductive system. The monitoring plan has to be rejected because it will not make essential data available.

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