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Testbiotech comment on the Scientific Opinion on an application by Dow AgroSciences LLC (EFSA-GMO-NL-2011-91) for the placing on the market of genetically modified herbicide-tolerant soybean DAS-68416-4 for food and feed uses, import and processing under Regulation (EC) No 1829/2003

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Introduction

Soybean DAS-68416-4 expresses the AAD-12 protein, which confers tolerance to 2,4-D and other related phenoxy herbicides, and the PAT protein which confers tolerance to glufosinate ammonium-based herbicides (EFSA, 2017a).

Molecular characterisation

The expression of the newly introduced proteins was only measured in field conditions in the US. It is unclear to which extent specific environmental conditions can influence the overall concentration of the newly introduced proteins 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 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 and, therefore, 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 focussing on comparative analysis and analysis of agronomic traits were conducted at eight locations in the US in 2009. No field trials were conducted in other soybean producing regions such as Argentina and Brazil.

Several differences were found in the comparison of agronomical and phenotypical traits. The criteria ‘days to 50% flowering’ fell under equivalence category III (non-equivalence is more likely than equivalence) for untreated soybeans.

Several significant changes were also found in the composition analysis:

• DAS-68416-4/untreated

Statistically significant differences from the conventional counterpart for 22 constituents (2 in forage and 20 in seeds).

The level of 18 of the 22 constituents fell under equivalence category I or II, while the level of four seed constituents fell under equivalence category III or IV.

• DAS-68416-4/2,4-D
Statistically significant differences were identified for 23 constituents (2 in forage and 21 in seeds). The level of 19 of the 23 constituents29 fell under equivalence category I or II, while the level of four seed constituents fell under equivalence category III or I.

• DAS-68416-4/glufosinate
Statistically significant differences were identified for 26 constituents (2 in forage and 24 in seeds). The level of 19 of the 26 constituents30 fell under equivalence category I or II, while the level of seven seed constituents fell under equivalence category III or IV (Table 3).

• DAS-68416-4/2,4-D + glufosinate
Statistically significant differences were identified for 20 constituents (1 in forage and 19 in seeds). The level of 16 of the 20 constituents31 fell under equivalence category I or II, while the level of four seed constituents fell under equivalence category III or IV.

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: moisture, stearic acid, calcium, four amino acids, iron content, folic acid, raffinose and lectin activity.

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.
- Investigation into changes in the content of miRNA that can be taken up from the gut and render biological effects across borders of life domains.
- Exposure of the plants to a wide range of defined biotic or abiotic stressors to assess the true range of possible changes in the plants´ composition
- Inclusion of more varieties inheriting the trait in order to investigate how the gene constructs interact with the genetic background of the plants.
- Feeding trials with the whole plants to assess potential health effects. The effects on the immune system were completely ignored in the assessment of potential health impacts from the 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 an acute toxicity study, a feeding study with chickens as well as three 28-day studies to confirm the safety of soybean DAS-68416-4. Two of the three 28-day studies were rejected by EFSA due to methodological flaws.

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

Despite biologically relevant differences being found in the comparative assessment, no further testing of the whole plant was requested. Strikingly, no 90-day subchronic study was requested by EFSA. This gap in risk assessment was criticised by experts from several EU Member States (EFSA, 2017b). Implementation Regulation (503/2013) requests 90-day subchronic studies are undertaken as part of the risk assessment for all applications filed after 2014. In the light of this regulation, it is obvious that such data also have to be requested in cases where many biologically relevant differences between the event and its comparator are found, including compounds such as lectins. Further, multigenerational studies should have been performed to assess the impact on the reproductive system.

Beyond this, the residues from spraying were considered to be outside the remit of the GMO panel. However, without detailed assessment of these residues no conclusion can be drawn on the safety of the imported products: 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 have 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. In addition, according to a reasoned legal opinion drawn up by Kraemer (2012), from a regulatory point of view, residues from spraying with complementary herbicides do, indeed, have to be taken into account in the risk assessment of genetically engineered plants.

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 spraying residues has to 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 avoid its obligation to make sure that the applicant provides all data necessary to assess the product derived from the soybean in all relevant health aspects.

There are good reasons to assess the residues from spraying with the complementary herbicides in detail:

- 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 DAS-68416-4 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 extended.[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 the interaction of residues from spraying with glufosinate and 2,4-D together.

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.

Consequently, the toxicological assessment as performed by EFSA is not acceptable.

Allergenicity

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

- 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 the risk for more vulnerable groups of the population, such as infants (EFSA, 2010), into account.
- The number of blood samples from patients with a known allergenicity to soybeans is too small to draw any conclusions.

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

2COMMISSION 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

- 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.

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 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.

References

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