TESTBIOTECH Background 09 - 05- 2017

Testbiotech comment on the Scientific opinion on an application (EFSA-GMO-NL-2013-120) for authorisation of genetically modified soybean FG72 x A5547-127 for food and feed uses, import and processing submitted in accordance with Regulation (EC) No 1829/2003 by Bayer CropScience LP and M.S. Technologies LLC

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[updated 4 July 2017]

Introduction

Soybean FG72 x A5547-127 produced by Bayer CropScience expresses three proteins (EFSA, 2017a):

- 2mEPSPS conferring tolerance to glyphosate-based herbicides,
- HPPD conferring resistance to isoxaflutole 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. However, the residues from spraying with the complementary herbicides were not assessed.

Molecular characterisation

Regarding parental events:

Soybean FG72 was produced using the ballistic method. As the application for soybean FG72 shows, several copies of the additional DNA inserted in the plants´ genomes revealed defragmentation and other unintended characteristics in the size and orientation of the copies. Risks were not assessed in depth by EFSA.

In soybean A5547-127, the gene construct was shown to be unintentionally divided into two parts; and parts of the DNA show reverse orientation and deletions. There were some open reading frames identified that can give rise to unintended gene products in the plants. Risks were not assessed in-depth by EFSA.

Regarding FG72 x A5547-127:

Differences in the expression of the HPPD protein were found between the single and the stacked event in measurements derived from field trials in the US (EFSA, 2017a):

> *"The reported expression levels of HPPD W336 in most tissues in the stack were lower than in the single (maximum ratio of twofold), although with overlapping ranges."*

Further, data generated in Brazil give a clear indication that the stacking of the event influenced the level of protein expression:

> *"Overall levels of HPPD W336 for both FG72 x A5547-127 and FG72 were low; levels of HPPD W336 in FG72 9 A5547-127 were below the method's limit of quantification, whereas in the single they were quantifiable."*

As a result, further investigations are necessary to assess the combinatorial genomic effects.

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

Agronomic characteristics

The data on agronomic characteristics also indicate genomic effects or – and this would also need to be assessed – genome x environmental interaction:

> *"However, for the endpoints stand count (early and final) and plant vigour the per-site summary statistics revealed that the observed differences can be mainly attributed to data derived from the sites where soybean FG72 x A5547-127 introgressed into MST39 was tested (maturity group 3 sites), where a reduction of ca. 50% was observed. Such reduction was not observed for the single events FG72 and A5547-127 previously assessed by the GMO Panel (EFSA GMO Panel, 2011c, 2015). The GMO Panel requested further information, but from the submitted data, it was not possible to fully characterise the observed differences."*

The results from the field trials should be assessed in more detail, looking at specific interactions between the additional DNA and the genetic background of the different plant varieties, as well as interaction between the environment and the genome.

Composition

Around half of the data assessed showed significant differences compared to the non-GM variety:

"The combination of test of difference and test of equivalence could be applied to the remaining 61 endpoints, with the following results:

- *Statistically significant differences between soybean FG72 9 A5547-127 (untreated) and the non-GM comparator were identified for 28 endpoints. 25 All the endpoints fell under equivalence category I.*
- *Statistically significant differences between soybean FG72 9 A5547-127 (treated) and the non-GM comparator were identified for 39 endpoints. 26 All the endpoints fell under equivalency category I."*

Although these differences were classified under category I, the high number of significant changes, in combination with the genomic effects and differences in the agronomic characteristics, should be investigated further.

But 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 from the gut and render biological effects across border of life domains (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.
- More varieties inheriting the trait should have been included to investigate how the gene constructs interact with the genetic background of the plants.

Further, as experts from Member States explain (EFSA, 2017b), due to the expression of the HPPD W336 protein, FG72 x A5547-127 and FG72 are resistant not only to isoxaflutole, which belongs to the isoxazoles, but possibly also to other herbicide families, e.g. triketones and pyroxazoles. In this respect, the transgenic trait has not been described comprehensively and the comparative assessment should possibly consider GM material that has also been treated with other herbicides.

As experts from Member States further explain (EFSA, 2017b), the plants were either treated, or not treated, with the three complementary herbicides in combination. It cannot be ruled out that the effects of isoxaflutole, glyphosate and glufosinate work against each other and cancel each other out - therefore, comparative assessment studies should also include GMO treated with each of the herbicides separately.

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

Toxicology
No feeding studies were presented with the whole food and feed:

- A subchronic 90-day feeding study of sufficient quality was provided for soybean FG72 but it suffered from major methodological deficiencies and was rejected by EFSA.
- No subchronic 90-day feeding study was provided for soybean A5547-127.
- No feeding study with stacked soybean FG72 x A5547-127 was presented.

Therefore, health risks stemming from feeding whole food and feed cannot be assessed. This kind of data should have been requested, especially in awareness of the high number of significant changes in compositional analysis, the effects observed on plant characteristics and the observed genomic effects.

Further, testing of whole food and feed is especially relevant for assessing potential health effects resulting from the combination of the residues from spraying with glyphosate, isoxaflutole and glufosinate.

The application of the complementary herbicides is part of regular agricultural practice in the cultivation of herbicide-resistant plants. Therefore, it can be expected that the residues from spraying will always be present in the harvest and could be seen as inevitable “constituents”.
In general, EFSA considers residues from spraying with the complementary herbicide to be outside the remit of the GMO panel. However, from a scientific and regulatory point of view, it cannot be justified that herbicide-resistant genetically engineered plants are assessed for health risks whilst at the same time residues from spraying with complementary herbicides are left aside. Health risk assessment cannot be reduced to what is required under Regulation 396/ 2005 (Pesticide Regulation) since this assessment does not take the specific pattern of exposure and relevant cumulative effects into account.

Due to the specific agricultural practices that go along with the cultivation of herbicide-resistant plants, there are specific patterns of applications, exposure and occurrence of specific metabolites and an emergence of combinatorial effects that require special attention. For example, large-scale commercial cultivation of these plants results in a strong selective pressure on weeds to develop

resistance to the herbicides (Sammons & Gaines, 2014). This problem is also relevant for health risk assessment, since it has led to increasing amounts of glyphosate being sprayed (Benbrook, 2016) and subsequently more residues in the harvest (Cuhra, 2015). Specific agricultural practices applied in the cultivation of these herbicide-resistant plants mean that, for example, specific patterns of applications, exposure, occurrence of specific metabolites and emergence of combinatorial effects all 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, residues of glufosinate, glyphosate and isoxaflutole, their metabolites and additives to the formulated product might accumulate and interact in the plants.

As a 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 are not taken into account in regular pesticide registration. Further, according to a reasoned legal opinion drawn up by Kraemer (2012), from a regulatory point of view, residues from spraying with complementary herbicides have to be taken into account in the risk assessment of genetically engineered plants.

In regard to the pending application, there are specific reasons for concern: Isoxaflutole is classified as “probably carcinogenic” (see: Reuter, 2015), or classified as a suspected human carcinogen (EFSA, 2016). Further, there are ongoing discussions about whether glyphosate is “probably carcinogenic” (IARC, 2015). 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.

In 2016, EFSA presented its peer review of the pesticide risk assessment of the active substance isoxaflutole (EFSA, 2016), which clearly shows major data deficiencies in regard to the requirements of Pesticide Regulation 396/2005. As a result, EFSA was unable to set MRLs for isoxaflutole and its metabolites for applications to genetically engineered soybeans.

Furthermore, in 2015, EFSA presented the results of the risk assessment of glyphosate. EFSA stated in its opinion (EFSA, 2015a) that there were not enough data available on the application of glyphosate to genetically engineered plants resistant to the herbicide. For this reason, EFSA was unable to deliver a conclusive risk assessment on the actual risks of residues from spraying with glyphosate and the various glyphosate formulations.

Consequently, the EFSA assessment cannot show that genetically engineered soybeans sprayed with isoxaflutole, glyphosate and glufosinate are safe.

In addition, there are many other substances such as oestrogens, allergens and antinutritional compounds present in the plants that in interaction with trait-related characteristics might act as stressors: There is a considerable amount of literature indicating that glyphosate formulations can act as so-called endocrine disruptors (see, for example, Thongprakaisang et al., 2013; Çağlar & Kolankaya, 2008; de Liz Oliveira Cavalli et al., 2013; Omran & Salama, 2013). Endocrine effects were found when young rats were exposed to soy milk in combination with glyphosate (Nardi et al., 2016). Since soybeans also produce a number of plant oestrogens with hormonal activity (de

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

[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

Lemos, 2001), there might be some synergistic or additive interaction with the residues from spraying with glyphosate formulations.

There are further relevant issues: For example, the potential impact on the intestinal microbiome also has to be considered. Such effects might be caused by the residues from spraying since glyphosate has been shown to have negative effects on the composition of the intestinal flora of cattle (Reuter et al., 2007) and poultry (Shehata et al., 2013). Further, Bremmer and Leist (1997) examined the possible conversion of NAG to glufosinate in rats. Up to 10% deacetylation occurred at a low dose of 3 mg/kg bw as shown by the occurrence of glufosinate in the faeces. The authors concluded, however, that most of the conversion was caused by bacteria in the colon and rectum although toxicity findings indicate partial bioavailability (Bremmer & Leist, 1997, see also EFSA 2017 (b)).

As a result, there is a huge 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 in the plants 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 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.

Allergenicity

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

- The residues from spraying might lead to adjuvant effects in the plant´s allergens.
- No non-IGE-mediated immune reactions were assessed although these effects must be considered relevant (Mills et al., 2013).
- The assessment did not take the risk for more vulnerable groups of the population, such as infants (EFSA, 2010), into account.
- No blood samples from patients with a known allergenicity to soybeans were investigated.

- 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 soybeans should be made available. Possible health impacts must be monitored in detail. Controls regarding residues from spraying with glyphosate, glufosinate and isoxaflutole 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, or 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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