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Testbiotech comment on the Scientific Opinion of EFSA on application EFSA-GMO-NL-2007-45 for the placing on the market of herbicide-tolerant, high-oleic acid, genetically modified soybean 305423 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Pioneer

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Introduction

Soybean 305423 contains a fragment of the gm-fad2-1 gene, which leads to changes in the oleic-acid composition of the product. Oleic composition of the soybean is changed by RNA interference. In addition, the gm-hra expression cassette confers tolerance to acetolactate synthase (ALS)-inhibiting herbicides, which include herbicides of the imidazolinone, sulfonylurea, triazolopyrimidine, pyrimidinyl(thio)benzoate and the sulfonylaminocarbonyltriazolinone chemical families. Soybean 305423 is approved for cultivation in the US and in Canada.

Molecular characterisation

Soybean 305423 contains two vector constructs:

- construct1 contains a fragment of a soybean gene designed to silence the expression of the endogenous fad2-1 gene. It alters the oleic acid composition of the bean, leading to a decreased level of the omega-6 fatty acid desaturase and a high-oleic acid phenotype
- construct 2 contains the gm-hra gene, which confers tolerance to ALS-inhibiting herbicides.

Soybean 305423 was produced by particle bombardment. This method is known to have a major impact on plant DNA (see for example Makarevitch et al., 2003). Molecular characterisation revealed multiple rearrangements and several complete and truncated copies of gene constructs were detected. These truncated DNAs and rearrangements can interfere with gene regulation in the plants and may cause unintended effects. Metabolic and genomic screening would be required to investigate such effects whereby environmental stress factors would also need to be taken into account. There have so far been no such investigations. Molecular characterisation has, however, revealed that one of the investigated plants showed signs of genetic instability.

The genetic modification to change the fatty acid composition in the soybeans is based on an inhibition of the expression of endogenous plants genes by RNAi interference (RNAi), resulting in reduced levels of the corresponding plant enzymes. The underlying molecular process is complex and encompasses the degradation of endogenous mRNAs. In this process, small interference RNA molecules might be produced, such as secondary (double stranded) dsRNAs, which can be biologically relevant to human health and the environment. (Short inhibitory) siRNA molecules may cause intended gene silencing and have off-target effects, i.e. may silence genes other than

those intended (Senthil-Kumar et al., 2011). These effects can be passed from the plant to humans or animals at the consumption stage. Potential biological effects will depend on similarities between the cell regulation in mammals and plants. Zhang et al. (2011) show such biological effects based on these similarities. Thus, for the risk assessment of plants that produce new dsRNA it is necessary to conduct bioinformatics studies to identify any likely unintended targets of the intended siRNAs in humans or animals.

For example, Heinemann et al. (2013) recommend the following process for a proper assessment of genetically engineered plants involving RNA interference:

> *„(1) bioinformatics to identify any likely, unintended targets of the dsRNA in humans and other key organisms; (2) experimental procedures that would identify all new intended and unintended dsRNA molecules in the GM product; (3) testing animal and human cells in tissue culture for a response to intended and unintended dsRNAs from the product; (4) long-term testing on animals; and possibly (5) clinical trials on human volunteers.“*

But no such studies were conducted.

Comparative analysis

The results of just one field trial (conducted in the US in 2011) were the basis for the comparative assessment. Given the complex nature of the genetic modification in soybean 305423, this is inadequate. Field trials should have been conducted in different climatic regions to investigate any possible genome x environment interactions. Further, there should have been systematic testing of the various groups of herbicides applied to the plants.

As experts from European Member States stated, field trials with soybean 305423 which were part of the original Pioneer dossier and which were not assessed by EFSA due to severe flaws in study design (a null segregant was used as control instead of the isogenic variety), had shown great differences in composition of soybean 305423 in different climatic regions.

The US field trial also showed significant differences in several compounds between soybean 305423, its isogenic counterpart and several other soybean varieties. There was no equivalence in 16 of 51 parameters in soybean 305423 not sprayed with ALS-inhibiting herbicides. Further, there was no equivalence in 16 of the 53 parameters in seeds from plants treated with ALS inhibitors.

Several significant differences can be attributed to the intended modifications in the fatty acid profile. However, apart from that, there were also changes in the levels of odd chain fatty acids. According to EFSA and Pioneer, the ALS enzyme may cause this unintended effect.

There were also significant differences (non-equivalence) in parameters such as calcium, zinc and glycitin and related total glycitein equivalents as well as in the trypsin inhibitor.
According to EFSA, the variation for glycitin in soybean even exceeded *„the lower and upper limits established by the non-GM reference varieties growing in the same field trial“*.
Further, there are some significant differences in the trypsin inhibitor, which might be caused by gene silencing. In addition, there were some significant differences in agronomic parameters.
Industry scientists in a recent study also confirmed some significant differences in yield. According to Spear et al. (2013), there was a significant yield drag in soybean 305423 when the construct was crossed in different genetic backgrounds:

> *„The results indicated that the negative impact of the transgene on seed yield was consistent across multiple genetic backgrounds, ...“*

However, EFSA saw no reason to ask for more data that might shed light onto the underlying mechanisms of the agronomic performance of soybean 305423.

Overall, soybean 305423 cannot be regarded as substantially equivalent. EFSA should have requested much more information on unintended genetic effects and possible metabolic changes. For example, a transcriptome and proteome analysis should have been performed to investigate unintended effects.

Toxicology

The applicant performed several nutritional studies with pigs, laying hens and broilers as well as a sub-chronic 90-day study with rats. Most of these studies are flawed and should have been excluded from the assessment:

- In the sub-chronic study with rats (Delaney et al., 2008) a negative segregant from soybean 305423 was used as control instead of the conventional counterpart; soybean 305423 in the feed was not treated with ALS-inhibitors;
- In the chicken study (McNaughton et al., 2008) the feeds were contaminated with another GM glyphosate-resistant soybean (a fact not mentioned by EFSA); soybean 305423 was not treated with ALS-inhibitors.

In general, nutritional studies on farm animals are of little value for the risk assessment. They are not sufficient to investigate the more subtle effects on human health that might be caused by the intended or unintended changes in the composition of the soybeans. In conclusion, there is practically no reliable data on possible toxicity and the effects on health from soybean 3054233.

Further, there is no information on residues from ALS-inhibiting pesticides or the metabolism of the various complementary pesticides and mixtures that can be applied to soybean 305423. According to Kleter et al. (2011), no herbicide metabolites could be detected in ALS-inhibitor-resistant soybeans. Kleter (2011) also states that there is only very limited knowledge on this subject (i.e. no studies on residues as established by the JMPR).

Allergenicity

EFSA (2010) speaks about the need for detailed investigations into allergenic risks for infants and individuals with impaired digestive functions. “The specific risk of potential allergenicity of GM products in infants as well as individuals with impaired digestive functions (e.g. elderly people, or individuals on antacid medications) should be considered, taking into account the different digestive physiology and sensitivity towards allergens in this subpopulation.” However, these specific risks were left aside during EFSA risk assessment.

Further, the soybeans were tested with sera from small groups of individuals known to react to allergens from soybeans. Several differences were observed but not deemed relevant. Instead, EFSA should have requested more detailed investigations. As the minutes of a meeting of the working group (WG) “Self Task on Allergenicity” of 24 September 2007 shows, EFSA has serious doubts about the reliability of the investigations with such a small number of patients conducted in this case. “More sera from patients are needed but they also need to be well characterised. Statistical calculations have been done showing that 60-70 well characterised sera are needed based on variability. Since this might not be feasible, the WG has to consider the reliability of studies with a lower number of sera.” Therefore,, the assessment conducted by EFSA is inadequate.

Nutritional Assessment
There are no data on the equivalence and quality of the products that are processed such as soybean sprouts, milk and baby food, or for products undergoing fermentation and heat treatment. Without such data, no conclusion can be drawn upon equivalence and food safety.

It is astonishing that there are no data on the effects of processing on compounds of soybean 305423. This is an obvious gap in risk assessment, which was also noted by the EU Commission in a different case (soybean MON87705). In the case of soybean MON87705, the Commission at least requested EFSA to conduct a separate assessment of the oil in this soybean event used for commercial frying (http://www.efsa.europa.eu/en/efsajournal/pub/3507.htm). It is hard to understand why EFSA once more omitted the assessment of the possible effects of processing.

Others
The assessment suffers from the fact that there is no independent data on soybean 305423. Even the studies that were published in peer-reviewed journals (such as the feeding studies) have Pioneer scientists among their authors. Therefore, industry influence on data cannot be excluded. Furthermore, much more data would be needed to assess true impact of these soybeans on human health. Conclusions cannot be made without detailed studies with human volunteers from various subgroups of consumers and all relevant processed food ingredients. This means that many more investigations are needed before these products could be marketed.

As a recent legal dossier compiled by Professor Ludwig Kraemer shows, the decision not to monitor effects on health at the stage when genetically engineered food is consumed, violates the requirements of EU regulations. Directive 2001/18 and Regulation 1829/2003 both require that potential adverse effects on human health from genetically modified plants are monitored during the use and consumption stage, including in those cases where such effects are unlikely to occur. Monitoring also has to include residues from spraying with the complementary herbicide. Thus, the EFSA opinion that monitoring of effects on health is unnecessary is wrong and contradicts current EU regulations.

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