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Testbiotech comment on the Scientific Opinion on application (EFSA-GMO-UK-2009-76) for the placing on the market of soybean MON 87769 genetically modified to contain stearidonic acid, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto

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Introduction

MON 87769 is a genetically engineered soybean with an altered fatty acid profile. This was achieved by inserting DNA from *Primula juliae* (primrose) and from *Neurospora crassa* (a fungus), neither of which have ever been part of the food chain. The newly produced proteins are involved in the desaturation of endogenous fatty acids into stearidonic acid. The gene insertion gives rise to four fatty acids (Stearidonic acid (SDA), gamma -linolenic acid (GLA) and two trans-fatty acids) and a reduction in linoleic acid (LA) and alpa-linoleic acid (ALA).

SDA is an omega-3 fatty acid which is a precursor of the long chain, poly-unsaturated omega-3 fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in humans and animals. For many years omega-3 fatty acids such as those found in fish oil and other sources were reported to have a positive effect on health. However, more recent epidemiological meta-studies were unable to prove that these products had any beneficial effect on health. (see for example Rizos, E.C, Ntzani E.E., 2014).

Monsanto plans to add oil derived from the soybeans to food products such as margarine, mayonnaise, shortening, salad dressings and ready-to-eat foods. In this context, Monsanto has filed patents for cakes, oil, margarine, and also for the usage of the soybeans in animal feed. It has, in addition, filed further patent applications on pork and on products from cattle, poultry and fish fed with the soybeans.

Once its products are on the market, it is likely that Monsanto will try to claim that its products have a beneficial effect on health. However, positive effects on health were neither discussed nor examined or proven in the EFSA assessment.

Molecular characterisation

New open reading frames were detected in the plants which can give rise to RNA that is translated into proteins or might be involved in gene regulation without producing proteins (RNAi). However, the open reading frames were not assessed in regard to non-coding RNA (miRNA, RNAi). RNAi mechanisms are relevant for risk assessment and might play a bigger role in unintended changes in the oil content and changes in metabolism of the plants observed (see below). miRNA might be transmitted to the consumer and there is dispute over whether it might interact with gene regulation in mammalian cells (see for example Zhang et al., 2011; Lukasik & Zielenkiewicz, 2014).

There was also no assessment of the expression of the constructs in the plants under conditions that could represent the true range of environmental conditions, or under conditions of stress such as that caused by ongoing climate change. This is amazing since existing data already show a high variability in the SDA content of the soybeans.

Further, there is no detailed description of the extent to which the native genes derived from its donors were technically changed and re-synthesised before being inserted into the soybeans.

Ultimately, a lot more data would have been needed for a sufficiently robust risk assessment. These data should have included information on the effects of the additional DNA on the plants genome, transcriptome, proteome and metabolome, and also taken a broad range of defined environmental stress conditions into account.

Further clarification is needed regarding an obvious mistake in the opinion. EFSA states that: „These bioinformatic analyses did not reveal the interruption of any known endogenous gene in the MON 88701 flanking regions.“ EFSA has confused soybean MON87769 here with soybean 88701.

Comparative analysis

According to the application, soybean MON 87769 differs from its conventional counterpart only in its fatty acid profile. This statement is not based on scientific findings. In fact, it cannot be denied, that - beyond the intended changes - the soybeans are not equivalent to the soybeans used as a comparator.

Various significant findings in the compositional analysis and agronomic performance were observed. The statistical analysis revealed increased protein and reduced carbohydrate content in seeds. These changes also concern the content of isoflavins, phytoestrogens and phytic acid which are relevant for risks to human health. For example, in one year, the content of soy-typical phytoestrogens (daidzein, genistein and glycitein) was lower in the GM variant and anti-nutrient (phytic acid) was increased in soybean MON 87769. New trans-fatty acids were also identified which are undesirable because of potential negative effects on health. Agronomic parameters such as lower yield were also observed.

The findings indicate unintended and undesirable changes in the metabolism of the soybeans and should have been a starting point for a much more detailed investigation into underlying mechanisms. However, instead of being subjected to a detailed consideration they were rejected and deemed irrelevant for food safety assessment.

In addition, an outdated statistical method (99 % tolerance level) was applied. EFSA dropped this method from the applicable guidance in 2011, due to its low statistical power. EFSA defended this decision by saying that the Monsanto application was filed before 2011:

> “The EFSA GMO Panel took into account the established tolerance intervals by the applicant for the comparative risk assessment when statistical significant differences between soybean MON 87769 and its conventional counterpart were observed. However, the latest EFSA guidance (2011) is referring to a different approach based on equivalence testing. This was not foreseen in the applicable EFSA guidance (2006) when application EFSA-GMO-UK-2009-76 was submitted.”

EU regulation 1829/2003 requires testing according to the highest scientific standards – so it is inexcusable to knowingly use statistical methods that are insufficient.

The conclusion must be that these differences should have been investigated in more detail, taking into account a broad range of defined environmental stress conditions. The assessment as performed by EFSA has to be rejected.

Food Safety Assessment

Toxicology

Monsanto seems to suggest that the usage of its soybean will be limited:

> "In order to derive commercial value from this product, the MON 87769 soybean crop will be grown and processed in an identity preserved manner in the northern US soybean growing regions and MON 87769 soybeans will be processed in dedicated oil processing facilities that will also be operated in an identity preserved manner and oil will be sold to food processors for food formulation."

However, Monsanto´s application is not restricted to specific purposes but covers all usage in food and feed. In this context, Monsanto has not only filed patents for cakes, oil and margarine, but also for usage of the soybeans in animal feed. For example, Monsanto has filed patent applications on pork (WO2009/073397) as well as on products from cattle, poultry and fish fed with the soybeans. These patents reveal that the company has a vast range of commercial interests that might become relevant once the soybeans are allowed on the market.

EFSA, on the other hand, only assessed very specific uses in some food products and deliberately omitted animal feed usage and changes in the composition of the animal products from animals fed with the soybeans. By doing so, EFSA failed to assess data available from feeding studies with pigs, cattle and fish which could be used to assess the effects of the soybeans on mammalian health in more detail (see WO 2010/107422,WO 2010/027788, WO 2009/097403, WO 2009/102558 and several publications). It is evident that EFSA is aware of these huge gaps in its risk assessment:

> "The EFSA GMO Panel notes that the quantitative dietary estimates described here would have to be revisited if the oil produced by soybean MON 87769 were to be extensively used in food products not considered in this assessment, for example as dietary supplements or to modify animal feed products."

Instead of requesting further investigation or at least taking note of existing data, EFSA accepted a 90 day animal feeding study with rats using only *defatted* soybeans in low quantities. No feeding study with the full-fat soybean was provided, while some feeding were performed with the oil on pregnant rats. The maximum duration of any study was around 120 days, which is much too short to assess potential effects on health. As EFSA in its answer to Member States notes:

> "Both hypothetical beneficial effects of a higher n-3 fatty acid intake and hypothetical adverse effects of a potentially somewhat higher intake of trans fatty acids are expected to take many years to evolve and are prone to be influenced by numerous confounders, which means that even a well-controlled long-term intervention study of a sufficient number of subjects is unlikely to provide a clear answer."

Furthermore, despite a request from EFSA, no toxicity study with the isolated proteins as produced in the plants was provided. In the opinion it says:

> "The Panel requested 28-day toxicity studies on the newly expressed proteins to confirm their safety in the absence of a history of consumption of these specific proteins. However, according to the applicant, it was not possible to generate sufficient protein preparations of

suitable quality."

There was a short term consumption study in humans, but the SDA used in the study was not derived from the soybeans and had a different structure and composition. Therefore this study does not have much value for the risk assessment of the genetically engineered soybeans in regard to composition, metabolites and interactions. For example, some new trans-fatty acids were observed in the soybeans that should have been taken into account (but were not assessed by EFSA at all). Such experiments should have been conducted over a much longer period of time and specific attention should have been given to susceptible individuals such as infants since the oil from the soybeans MON87769 might be used in baby milk products. As a expert from the Member States notes:

> „these fatty acids and their elongation products interact with each other, possibly influencing eicosanoid metabolism and levels of the different eicosanoids which are physiologically very active, there is a remote possibility that in some circumstances or some individuals the use of MON 87769 derived products may have negative effects. It is suggested that some clinical experiments are done in human volunteers using SDA oil (e.g. determination of hemostatic factors)."

Consequently, the toxicity testing is not conclusive and leaves too many uncertainties.

Allergenicity

Testing of susceptible individuals for allergenic risk was only done on a very small number of samples so that no conclusions can be drawn. EFSA did admit this deficiency.
In addition, methods such as the pepsin test used to assess the allergenic potential of the proteins are known to be unreliable.

Neither does the EFSA approach take potential adjuvant / synergistic effects into account. No non-IGE-mediated immune reactions were taken into account, although these effects have to be considered relevant (Mills et al., 2013).

EFSA should have been pointing out that the existing data are simply not sufficient to derive sufficient evidence. For example, EFSA (2010) requests 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 completely left aside during EFSA's risk assessment. Ignoring the high level of uncertainties, EFSA is concluding:

> "The EFSA GMO Panel considers that there is no evidence that the genetic modification might significantly change the overall allergenicity of soybean MON 87769 when compared with that of its conventional counterpart."

Nutritional assessment

It is astonishing that the claims made by Monsanto on the benefits to health have not been assessed by EFSA at all. Monsanto expressly states that the claims regarding benefits to health are included in the application. Clearly as such they should have been assessed by EFSA:

> “Recommendations to increase consumption of long chain omega-3 polyunsaturated fatty acids have been made by a number of world-wide government and public health agencies and scientific organisations. Although the benefits of omega-3 fatty acid consumption are widely recognised, typical Western diets contain very little fish, and the dietary intake of omega-3 fatty acids is generally quite low relative to recommended intake. An alternative approach to increase omega-3 fatty acid intake is to provide a wider range of foods that are enriched in omega-3 fatty acids so that people can choose foods that suit their usual dietary habits. The oil derived from MON 87769 (SDA soybean oil) contains increased levels of SDA (approximately 20-30%) and GLA (~7%) and can serve as an alternate sustainable source of omega-3 fatty acid and help meet the need for increased dietary intake of long chain omega-3 fatty acids.”

But EFSA did not even mention potential effects on health. There was no review of existing literature or discussion of potential negative effects on health from a higher intake of omega 3 fatty acids (see for example Chua et al., 2013, see also www.nhs.uk/news/2013/07July/Pages/fish-oil-supplements-linked-to-prostate-cancer.aspx).

Long term epidemiological studies would be necessary to gain more reliable data. But as the existing discussion on existing epidemiological studies show, it might be hard to achieve the necessary clarity. EFSA is also aware of the problem and states that (as quoted above):

> “Both hypothetical beneficial effects of a higher n-3 fatty acid intake and hypothetical adverse effects of a potentially somewhat higher intake of trans fatty acids are expected to take many years to evolve and are prone to be influenced by numerous confounders, which means that even a well-controlled long-term intervention study of a sufficient number of subjects is unlikely to provide a clear answer.”

So why did EFSA not ask for a lot more data to at least lower the level of uncertainties and close some of the most evident gaps in its risk assessment? Why did EFSA not deal with long term effects on health at all? It looks like the opinion of EFSA is driven by a profound bias in favor of the applicant. In consequence, crucial data and investigations were not requested, fundamental uncertainties were not given enough emphasis.

In this context also a statement made by EFSA in response to comments from experts of Member States has to be discussed in detail:

> “The Panel agrees in principle to the concept that MON 87769 soybean oil could or should replace other vegetable oils, including conventional soybean oil, added to processed foods. The Panel agrees to the MS statement that MON 87769 soybean oil is needed to achieve an optimal dietary fatty acid pattern because this is possible with a combination of foods with appropriate fatty acid patterns.”

This statement that reads like an advertisement for the commercial interests of Monsanto (and might even be used by the company in future) should be a reason for Member States and the EU Commission to urgently ask for clarification. At the moment it can not be excluded that the meaning of this sentence was confused by typing errors. If so, it has to be corrected. If not, this statement definitely should be a reason for major revision in the composition of the GMO panel.

In any case it is evident that EFSA´s risk assessment as applied in genetically engineered plants lacks an adequate approach to deal with more subtle long term effects on health. This EFSA opinion indicates that standards required under the Novel Food regulation or standards applied by EFSA in relation to benefits to health can be avoided if the relevant product is filed under GMO regulation.

Others

As a legal dossier compiled by Professor Ludwig Kraemer shows, EU regulations require the monitoring of effects on health at the stage of consumption. This is especially relevant in this case, because possible negative effects on health are only likely to be detected in long-term observations. 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. Certainly, in this case, the EFSA opinion that monitoring the effects on health is unnecessary contradicts current EU regulations.

Conclusions and recommendations

The risk assessment is inconclusive, is likely to be driven by fundamental bias and in any case does not answer the decisive questions arising from potential health claims for this product. Market authorisation for import and usage in food and feed cannot be given. In general, EFSA´s risk assessment lacks an adequate approach to deal with more subtle long term (positive or negative) effects on health.

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