

GeneWatch UK's response to EFSA's scientific opinion on an application for the placing on the market of food, feed and other products containing or consisting of herbicide tolerant, increased oleic acid genetically modified soybean 305423 x 40-3-2 and food and feed produced from this soybean.

Comments

a. Assessment:

Molecular characterisation

The molecular characterisation is acknowledged to be unusually complex, including complete and/or partial copies of the cassettes in 4 different insertion arrangements plus an unintended fragment (claimed to be non-functional). The applicant has also demonstrated instability in the genome of soybean 305423 as a single plant has been found to be GM-HRA negative. Further, the use of RNA interference can give rise to unintended off-target effects (Heinemann JA, Agapito-Tenfen SZ, Carman JA. A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. *Environment International*. 2013;55:43–55; 1. Lundgren JG, Duan JJ. RNAi-Based Insecticidal Crops: Potential Effects on Nontarget Species. *BioScience*. 2013;63(8):657–665. doi:10.1525/bio.2013.63.8.8). Especially given the unexpected and unintended alterations in compositional analysis (e.g. altered calcium, zinc, glycitin, trypsin inhibitor and forage fibre fractions, as well as complex and unexpected effects on fatty acid profile), a full proteomic analysis should be requested from the applicant. Such an analysis would be able to better characterise these unintended effects (Zolla L, Rinalducci S, Antonioli P, Righetti PG. Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications. *J Proteome Res*. 2008;7(5):1850–1861).

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

Field trials at North American sites only are insufficient to provide the necessary data, particularly for nutritional analysis. Environment and gene-environment interactions (GxE) are known to have important effects on nutrient (including fatty acid) composition of soybeans (Whent M, Hao J, Slavin M, et al. Effect of Genotype, Environment, and Their Interaction on Chemical Composition and Antioxidant Properties of Low-Linolenic Soybeans Grown in Maryland. *J Agric Food Chem*. 2009;57(21):10163–10174) and such effects can vary at different developmental stages (Han Y, Xie D, Teng W, Zhang S, Chang W, Li W. Dynamic QTL analysis of linolenic acid content in different developmental stages of soybean seed. *Theor Appl Genet*. 2011;122(8):1481–1488). It is therefore essential that data is obtained from a wide variety of agronomic conditions, representative of expected growing conditions. In the interests of achieving a level regulatory playing field it is also worth noting that Monsanto included data from the US and Chile in its MON87705 (Vistive Gold) application (EFSA, 2012). Whilst the MON87705 data is arguably also insufficient, soybean 305423 x 40-3-2 contains worrying signs of unintended effects on nutrient composition which should warrant more data being supplied not less. Further data from other sites (including South America) and different years should be requested from the applicant.

Rather than proceeding with what it states is a suboptimal comparator, EFSA should require the correct comparator to be used.

b. Food Safety Assessment:

Toxicology

The product is genetically engineered to be tolerant to two classes of herbicide: ALS inhibiting herbicides and glyphosate. The only rat feeding study supplied by the applicant (Qi et al., 2012) is acknowledged by the EFSA GMO Panel to be inadequate, but they wrongly did not request a better trial. However, one aspect not acknowledged by the EFSA GMO Panel is the lack of information regarding whether the GM soybeans in the study were treated with herbicides, especially whether they were blanket-sprayed with glyphosate, as intended for this glyphosate-tolerant crop, and whether any other herbicides were used such as ALS inhibiting herbicides. Since the study does not report use of herbicides, it may be assumed they were not used and thus the effects of pesticide residues have not been tested.

For glyphosate-resistant GM crops, application of glyphosate alters the nutrient profile as well as leaving pesticide residues on the soybeans (Bellaloui N, Abbas HK, Gillen AM, Abel CA. Effect of glyphosate-boron application on seed composition and nitrogen metabolism in glyphosate-resistant soybean. *J Agric Food Chem.* 2009;57(19):9050–9056.; Bøhn T, Cuhra M, Traavik T, Sanden M, Fagan J, Primicerio R. Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. *Food Chemistry.* 2014;153:207–215). It is therefore essential to include a study of the actual product as it is intended to be produced, with the intended herbicide. This is particularly important for the soybean oil as this is the product intended to be fed to humans.

When grown as intended, as a herbicide-tolerant crop, the soybeans are likely to contain higher residues of these herbicides than conventionally grown soybeans due to blanket spraying: Arregui, M. C., Lenardón, A., Sanchez, D., Maitre, M. I., Scotta, R., & Enrique, S. (2004). Monitoring glyphosate residues in transgenic glyphosate-resistant soybean. *Pest Management Science*, 60(2), 163–166.

Bohm, G.M.B., Rombaldi, C. V., Genovese, M. I., Castilhos, D., Alves, R., ... Rumjanek, N. G. (2014). Glyphosate Effects on Yield, Nitrogen Fixation, and Seed Quality in Glyphosate-Resistant Soybean. *Crop Science*, 54(4), 1737–1743. <http://doi.org/10.2135/cropsci2013.07.0470>

Bøhn, T., Cuhra, M., Traavik, T., Sanden, M., Fagan, J., & Primicerio, R. (2014). Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. *Food Chemistry*, 153, 207–215.

However, potential adverse health effects of these residues have not been considered. These include adverse effects of each pesticide singly, and in combination, and the effects of additives in commercial formulations (as well as the main active ingredient).

New evidence regarding carcinogenicity of glyphosate has been ignored:

Guyton, K. Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Scoccianti, C., Mattock, H., Straif, K. (2015). Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *International Agency for Research on Cancer*.

Effects of additives have been ignored, see e.g.:

EFSA, European Food Safety Authority, (2015) Statement of EFSA on the request for the evaluation of the toxicological assessment of the co-formulant POE-tallowamine. *EFSA Journal* 2015;13(11):4303, 13 pp.

Cox, C., & Surgen, M. (2006). Unidentified Inert Ingredients in Pesticides: Implications for Human and Environmental Health. *Environmental Health Perspectives*, 114(12), 1803–1806.

Benachour, N., & Séralini, G.-E. (2009). Glyphosate Formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic, and Placental Cells. *Chemical Research in Toxicology*, 22(1), 97–105.

Mesnage, R., Bernay, B., & Séralini, G.-E. (2013). Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. *Toxicology*, 313(2–3), 122–128.

Mesnage, R., Defarge, N., Vendomois, J. S., Séralini, G.-E. (2014). Major Pesticides Are More Toxic to Human Cells Than Their Declared Active Principles. *BioMed Research International*, 2014, e179691.

Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., & Seralini, G.-E. (2005). Differential Effects of Glyphosate and Roundup on Human Placental Cells and Aromatase. *Environmental Health Perspectives*, 113(6), 716–720.

Young, F., Ho, D., Glynn, D., Edwards, V. (2015). Endocrine disruption and cytotoxicity of glyphosate and roundup in human Jar cells in vitro. *Integrative Pharmacology, Toxicology and Genotoxicology*. Vol. 1(1): 12-19.

Hormone disruption effects of pesticide residues have also been ignored e.g.

Thongprakaisang, S., Thiantanawat, A., Rangkadilok, N., Suriyo, T., & Satayavivad, J. (2013).

Glyphosate induces human breast cancer cells growth via estrogen receptors. *Food and Chemical Toxicology*, 59, 129–136.

Abarikwu, S. O., Akiri, O. F., Durojaiye, M. A., & Adenike, A. (2015). Combined effects of repeated administration of Bretmont Wipeout (glyphosate) and Ultrazin (atrazine) on testosterone, oxidative stress and sperm quality of Wistar rats. *Toxicology Mechanisms and Methods*, 25(1), 70–80.

Regulation requires that synergistic and combined effects of herbicides must be considered.

However, the synergistic effects of the multiple herbicides to be used in combination with this herbicide-resistant crop have not been considered.

Allergenicity

Nutritional assessment

There is no nutritional assessment as such included in the scientific assessment and the EFSA GM Panel appears to be relying solely on The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)'s 2010 report on Dietary Reference Values for fatty acids. This was also the case for the single soybean 305423 event.

GeneWatch UK considers the lack of any proper nutritional assessment to be a serious omission from the scientific assessment. Combined with the lack of adequate labelling (see below) it means that in practice, consumers will have no idea about the nutrient content of the foods they are consuming. Potentially serious safety issues could be missed and there is no clear mechanism for recall of products if (as is common in the nutrition literature) new studies identify unexpected adverse effects or confirm adverse effects that are currently uncertain, some of which may impact the health of specific subpopulations.

Use of the NDA Dietary Reference Values (DRVs) is inadequate for a number of reasons including: (i) the report is out of date and more recent studies must be included in the scientific assessment of soybean 305423; (ii) it does not consider population subgroups who may be particularly affected by

changes in the fatty acid profile of their food; (iii) it is not applicable to GMO foods which require a safety assessment under Regulation (EC) No. 1829/2003. This requires a scientific evaluation of the highest possible standard (conducted by EFSA) followed by a risk management decision by the Community.

The introduction of GM soybean oil with altered nutritional properties onto the EU market is a decision which is the responsibility of EU institutions, not merely a recommendation (as DRVs are) to individuals about what foods to consume. GM foods placed on the market in the EU must not have adverse effects on human health or be nutritionally disadvantageous for the consumer (EC 1829/2003 Article 4(1)) and no authorisation can be granted unless the applicant has adequately and sufficiently demonstrated this. A full nutritional assessment is therefore required by EFSA. This should not have been omitted.

This was an issue for the soybean 305423 dossier, but it is compounded by the fact there are some significant unexplained differences in fatty acid composition between the stacked event and the single event, as reported in Section 4.3.1, as well as some unexpected effects (increases in odd chain fatty acids) which also occur in soybean 305423. We refer to our previous comments on this issue: http://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/consultation_form_soybean305423.pdf

Both linoleic and alpha-linolenic acids are significantly reduced in the GM soybeans. It is a step forward (compared to the analysis for the single trait) that the risk of linoleic deficiency has been considered for the current application, however there are some major limitations in the approach used which render it inadequate. These include: (i) use of UK data only to represent EU consumers with a wide range of dietary habits; (ii) use of (old) DRV's (from 2010), as noted above; (iii) lack of consideration of vulnerable subpopulations.

As well as those with genetic disorders (see link to previous submission), vulnerable subgroups may include unborn children as "Essential fatty acids" are among the most important fatty acids during the intrauterine growth period. These are α -linolenic acid, which is a precursor of the n-3 series, linoleic acid, which is a precursor of the n-6 series and their derivatives, represented by docosahexaenoic acid and arachidonic acid. The latest studies have shown that medium-chain fatty acids also play a significant role in maternal-fetal metabolism. See: Bobiński, R., & Mikulska, M. (2015). The ins and outs of maternal-fetal fatty acid metabolism. *Acta Biochimica Polonica*, 62(3), 499–507.

Another important subgroup may be autistic children, as linolenic and linoleic acids below the 5th percentile of the control values, were found in 43% and 38% of autistic children respectively in a recent study: Mostafa, G. A., & AL-Ayadhi, L. Y. (2015). Reduced levels of plasma polyunsaturated fatty acids and serum carnitine in autistic children: relation to gastrointestinal manifestations. *Behavioral and Brain Functions* : BBF, 11. <http://doi.org/10.1186/s12993-014-0048-2>

Further, this issue should also have been considered for animals supplied with the GM soybeans as feed. For example, Rosero et al. (2016) conclude that "*a minimum dietary intake of 10 g/d of α -linolenic acid, simultaneous with a minimum of 125 g/d of linoleic acid should be provided to ≥ 95 % of the sows; thereby, achieving a maximum sow reproductive efficiency through multiple mechanisms that include rapid return to estrus, high maintenance of pregnancy and large subsequent litter size in mature sows, that appear to be susceptible to EFA deficiency*". Rosero, D. S., Boyd, R. D., Odle, J., & van Heugten, E. (2016). Optimizing dietary lipid use to improve essential fatty acid status and reproductive performance of the modern lactating sow: a review. *Journal of Animal Science and Biotechnology*, 7, 34.

The applicant has applied for an authorisation which covers the GMO and foods containing it. Although information on the nutritional composition has been supplied for the GMO, it has not been supplied for the foods containing it. This means that no assessment can be conducted for such foods and no authorisation can be granted. Data on the nutrient (and anti-nutrient) composition of all the foods within the scope of the application (salad dressings, margarines, cooking oils, salty snacks, tofu, soymilk etc.) must be provided by the applicant as well as for secondary products such as soy lecithin.

Nutrient (and anti-nutrient) composition is also required for meat, milk and eggs from animals fed on soybean 305423 x 40-3-2. The scientific assessment incorrectly implies that the soybean oil will be largely for human consumption, whilst defatted soybean meal will be fed to animals. Whilst this is indeed normal practice in the industry, the addition of GM soybean oil or seeds to animal feed is an active topic of research, with the aim of altering milk fat composition (Bernal-Santos G, O'Donnell AM, Vicini JL, Hartnell GF, Bauman DE. Hot topic: Enhancing omega-3 fatty acids in milk fat of dairy cows by using stearidonic acid-enriched soybean oil from genetically modified soybeans. *J Dairy Sci.* 2010;93(1):32–37. doi:10.3168/jds.2009-2711) as has already been attempted using supplements (e.g. Glasser F, Ferlay A, Chilliard Y. Oilseed lipid supplements and fatty acid composition of cow milk: a meta-analysis. *J Dairy Sci.* 2008;91(12):4687–4703). Since potential food and feed applications have not been restricted, this application should fall within the scope of the assessment. Further, it is likely that a similar approach could be applied to meat and eggs where diet is known to affect fat composition (e.g. Berthelot V, Bas P, Schmidely P. Utilization of extruded linseed to modify fatty composition of intensively-reared lamb meat: effect of associated cereals (wheat vs. corn) and linoleic acid content of the diet. *Meat Sci.* 2010;84(1):114–124.; Oliveira DM, Ladeira MM, Chizzotti ML, et al. Fatty acid profile and qualitative characteristics of meat from zebu steers fed with different oilseeds. *J Anim Sci.* 2011;89(8):2546–2555).

Although a limited quantity of oil was included in the chicken feeding study (0.5%) this is insufficient to explore the possible deliberate application of a greater quantity of oil with the intention of altering the fatty acid profile of the eggs (so that they can potentially be marketed as premium products like “omega-3 eggs”). Further, no data on the nutrient profiles of the eggs has been reported. This is necessary for the nutritional assessment. Additional data should be requested from the application to cover these scenarios, to underpin a revised nutritional assessment.

Others

3. Environmental risk assessment

More weight should have been given to the concern that feral GM plants could survive if exposed to relevant herbicides, including ALS-inhibitors and glyphosate, which is very widely used.

4. Conclusions and recommendations

The risk assessment is incomplete and inadequate to support approval of the product.

5. Others

6. Labelling proposal

For specific labelling, the applicant proposed that, for example, operators handling products containing or consisting of oil produced from soybean 30542 x 40-3-2 shall be required to label these products with the words 'genetically modified soybean with altered fatty acid profile'. EFSA has accepted this, but it is totally inadequate.

Numerous GM soybeans with altered fatty acid profiles are in the GM industry pipeline with a wide variety of properties (http://www.soyconnection.com/sites/default/files/Biotech_PipelineCharts.pdf and Wilson RF. The role of genomics and biotechnology in achieving global food security for high-oleic vegetable oil. *J Oleo Sci.* 2012;61(7):357–367). These products all have different fatty acid profiles and molecular characterisations (see for example the EFSA Scientific Opinion on MON88705). It is essential that consumers and medical professionals are provided with more information on the label (i.e. a list of all fatty acids and other nutrients that are significantly increased or decreased) and the means to find more detailed information should this become necessary (i.e. the Unique Identifier). This is essential because:

1. New information may become available in future about unexpected harms associated with the particular method of genetic modification or molecular characterisation (e.g. stability of a particular construct or off-target effects) which is only traceable via the Unique Identifier.
2. New information may become available regarding specific harms associated with specific types of fatty acid (e.g. confirming the reported association between omega-3 fatty acids and prostate cancer) which may lead to (some or all) consumers wishing to avoid some altered oil products but not others and/or retailers/manufacturers to withdraw some products. This can only be done if the fatty acid profile of each product is known and its source is traceable.
3. Small subgroups of consumers (e.g. suffering from a particular metabolic disorder) may find health problems are caused by some fatty acid profiles but not others. They may therefore wish (or need) to avoid specific fatty acids or groups of fatty acids.

Any of these situations may necessitate withdrawal of products and/or consumer information to be issued regarding specific products (allowing specific subgroups of persons to avoid them). This can only be done if the fatty acid profile and its source is known to the consumer (and in some cases can be discussed with a medical professional) via information on its label.

Regulation (EC) 1829/2003 Preamble (22) states:

"In addition, the labelling should give information about any characteristic or property which renders a food or feed different from its conventional counterpart with respect to composition, nutritional value or nutritional effects, intended use of the food or feed and health implications for certain sections of the population, as well as any characteristic or property which gives rise to ethical or religious concerns".

The proposed labelling does not conform to these requirements. A new proposal is therefore needed.

Although not currently provided for in the legislation, labelling of meat, milk and dairy products from animals fed on soybean 305423 x 40-3-2 as feed is also necessary, because the use the potential use of whole soybeans or soybean oil as dietary supplements can significantly alter the fatty acid profile of these products.