

GeneWatch UK comments on the Opinion of the Scientific Panel on Genetically Modified Organisms on an application for the authorisation of GM soybean 87769 for food and feed uses submitted under Regulation (EC) No. 1829/2003 by Monsanto

1. Sender: GeneWatch UK

2. Publication of the comments: Yes

3. Comments

a. Assessment:

Molecular characterisation

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

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.

Data on agronomic and phenotypic characteristics of soybean MON 87769, its conventional counterpart and a set of non-GM commercial varieties were collected in field trials performed in the USA in 2006 and 2007. These field trials also supplied seed and forage material for compositional analysis of the various soybean materials. In both years, the field trial was carried out at five geographical sites representative of the soybean cultivation areas of the USA. It is questionable whether this data set is sufficient to establish variability of nutrient levels between different sites and growing conditions. More data should be requested from the applicant.

b. Food Safety Assessment:

Toxicology

Soybean MON 87769 contains a single insertion consisting of two intact expression cassettes (*Pj.D6D* and *Nc.Fad3*) coding for the fatty acid $\Delta 6$ desaturase from *Primula juliae* (primrose) (*Pj.D6D*) and the fatty acid $\Delta 15$ desaturase from *Neurospora crassa* (red bread mold, a filamentous fungus) (*Nc.Fad3*).

The newly expressed desaturases in soybean MON 87769 seeds result in an alteration of the fatty acid profile, leading to the appearance of four new fatty acids (stearidonic acid (SDA), also known as octadecatetraenoic acid; alpha-linolenic acid; and two trans-fatty acids, 9c,12c,15t trans-ALA (18:3) and 6c,9c,12c,15t trans-SDA (C18:4)) and a reduction in linoleic acid (LA).

SDA is a normal intermediate in the formation of the long chain omega-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid [(C20:5 (n-3)) (EPA) and docosahexaenoic acid [(C22:6 (n-3)) (DHA). However, in humans, the conversion of ALA to SDA is slow. Direct consumption of SDA avoids this

step in the biosynthesis and the Opinion states that the rationale for developing the product is that this may result in a more efficient synthesis of the higher chain-length PUFAs (EPA and DPA).

There is limited evidence of this from a study conducted by Monsanto and Southern Illinois University in rats (Casey, J. M., Banz, W. J., Krul, E. S., Butteiger, D. N., Goldstein, D. A., & Davis, J. E. (2013). Effect of stearidonic acid-enriched soybean oil on fatty acid profile and metabolic parameters in lean and obese Zucker rats. *Lipids in Health and Disease*, 12, 147. doi:10.1186/1476-511X-12-147). After 12 weeks, SDA oil from the GM soybean raised omega-3 index (EPA + DPA) slightly more than the flax diet, but less than the fish diet. No studies in humans appear to have been conducted.

Apart from the limited evidence of efficacy in raising the omega-3 index, the entire rationale for the product appears to be based on the false assumption that long-chain omega-3 PUFAs are of benefit for health: this is not supported by current scientific evidence.

A 2009 Cochrane Review found *“There is not enough evidence to say that people should stop taking rich sources of omega 3 fats, but further high quality trials are needed to confirm the previously suggested protective effect of omega 3 fats for those at increased cardiovascular risk”* (Summary on: <http://summaries.cochrane.org/CD003177/there-is-not-enough-evidence-to-say-that-people-should-stop-taking-rich-sources-of-omega-3-fats-but-further-high-quality-trials-are-needed-to-confirm-the-previously-suggested-protective-effect-of-omega-3-fats-for-those-at-increased-cardiovascular#sthash.zuV7fUQ6.dpuf>). See also: Hooper, L., Thompson, R. L., Harrison, R. A., Summerbell, C. D., Ness, A. R., Moore, H. J., ... Davey Smith, G. (2006). Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. *BMJ (Clinical Research Ed.)*, 332(7544), 752–760. doi:10.1136/bmj.38755.366331.2F).

More recently, a meta-analysis by Rizos et al. (2012) concludes: *“Overall, omega-3 PUFA supplementation was not associated with a lower risk of all-cause mortality, cardiac death, sudden death, myocardial infarction, or stroke based on relative and absolute measures of association”*. (Rizos EC, Ntzani EE, Bika E, Kostapanos MS, & Elisaf MS. (2012). Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: A systematic review and meta-analysis. *JAMA*, 308(10), 1024–1033. doi:10.1001/2012.jama.11374). And another by Kwak et al. (2012) states: *“Our meta-analysis showed insufficient evidence of a secondary preventive effect of omega-3 fatty acid supplements against overall cardiovascular events among patients with a history of cardiovascular disease”*.

The findings of lack of benefit are supported by randomized controlled trials reported by Fezeau et al.: *“These results, as the lack of impact of our supplementation trial with DHA-EPA on CVD recurrence [18], do not support the recommendations of use of n-3 PUFA for the secondary prevention of CVD”* (Fezeu, L. K., Laporte, F., Kesse-Guyot, E., Andreeva, V. A., Blacher, J., Hercberg, S., & Galan, P. (2014). Baseline Plasma Fatty Acids Profile and Incident Cardiovascular Events in the SU.FOL.OM3 Trial: The Evidence Revisited. *PLoS ONE*, 9(4). doi:10.1371/journal.pone.0092548).

Whilst lack of efficacy is not an issue for a risk assessment as such, it remains uncertain whether increasing omega-3 PUFAs, as the manufacturer intends, is in reality beneficial or harmful for health. For example, studies suggesting a link between omega-3 fatty acids and prostate cancer have been ignored (Brasky TM, Darke AK, Song X, et al. Plasma phospholipid fatty acids and prostate cancer risk in the SELECT trial. *J Natl Cancer Inst*. 2013;105(15):1132–1141; Brasky TM, Till C, White E, et al. Serum Phospholipid Fatty Acids and Prostate Cancer Risk: Results From the Prostate Cancer Prevention Trial. *Am J Epidemiol*. 2011;173(12):1429–1439; Chua ME, Sio MCD, Sorongon MC, Morales ML Jr. The relevance of serum levels of long chain omega-3 polyunsaturated fatty acids and

prostate cancer risk: A meta-analysis. *Can Urol Assoc J.* 2013;7(5-6):E333–343). This risk cannot be assessed with the limited data provided in the dossier.

Further, the actual changes in composition are much more complex and substantive than a simple increase in SDA. In both years of field trials the most notable changes were a reduction in linoleic acid from 52.4–56.0 % to 16.5–30.8 % and in oleic acid from 17.2–21.5 % to 12.7–19.8 % of total fatty acids. This reduction was accompanied by the appearance of the two metabolites SDA (16.8–33.9 %) and GLA (6.1–8.0 %). In addition, low amounts of two trans-fatty acids previously not found in measurable concentrations in soybean oil, 9c,12c,15t trans-ALA (18:3) at 0.15–0.48 % and 6c,9c,12c,15t trans-SDA (C18:4) at 0.06–0.26 %, were detected. Some other statistically significant alterations in composition were found in some or all batches (e.g. vitamin E, protein, phytase). The impacts of making these very significant changes in the human diet have not been assessed at all.

Soybeans were harvested from two of the five sites in the USA in 200624 in order to perform compositional analyses on processed fractions, including defatted and toasted meal; refined, bleached and deodorised oil; protein isolate; and crude lecithin, derived from MON 87769, A3525 and eight conventional reference soybean varieties. Comparing the defatted and toasted meal produced from soybean MON 87769 with similar meals from the conventional counterpart showed changes in the fatty acid profile that mirrored the differences seen with the whole soybean (i.e. reduced LA and oleic acid and the appearance of SDA and GLA). Statistically significant changes in the concentration of six other constituents were also found. When the compositional data on processed oils from both types of soybean were compared (Table 1), statistically increased levels of palmitic acid, stearic acid, trans-ALA and vitamin E were observed, whereas the level of lignoceric acid was reduced. The level of LA was extensively reduced (from 54.8–55.9 % in the conventional counterpart to 20.7–30.9 % of the fatty acids in soybean MON 87769). In addition to these changes, three of the new fatty acids identified in the whole seed were also seen in the refined oil from MON 87769 (SDA, GLA and trans-SDA, constituting 16.9–28.4 %, 6.2–7.2 % and 0.17–0.39 % of the total fatty acids respectively). Small quantities of trans-ALA were present in all types of refined, bleached and deodorised soybean oil, suggesting that small quantities of this trans-fatty acid may be produced during processing of the oil. Owing to the lack of commercially available standards for 9c,12t,15c, 9t,12c,15t and 9t,12c,15c C18:3 trans-fatty acids, these could not be individually quantified. The crude lecithin derived from soybean MON 87769 contained SDA, GLA and trans-SDA, which are usually not detected in lecithin from conventional soybeans. In lecithin, the level of linoleic acid (C18:2) was reduced from 57.3–58.7 % of total fatty acids in soybean A3525 to 22.1–34.3 % of total fatty acids in soybean MON 87769.

No analysis has been provided of the fatty acid of the final products for which the applicant is seeking approval (e.g. salad dressings and margarines, or products fried in the oil).

Based on the results of short-term studies in rats, the Opinion concludes that feeding stuffs derived from defatted soybean MON 87769 are as safe and nutritious as those derived from other non-GM soybean varieties. However, this completely ignores the expected metabolic effects such as raising omega-3 PUFAs, which have not been assessed at all.

The opinion cites intervention studies on humans with various amounts of SDA ethyl esters and/or SDA-containing plant derived oils, and with SDA-enriched soybean oil for between 14 and 84 days and at doses ranging from 0.05 to 4.2 g SDA/day, stating no adverse effects were reported. But such studies are wholly inadequate to assess long-term effects such as cancer risk.

Similarly, several studies cited in which human diets were supplemented with GLA at doses from 1 to 5 g/day for periods of one to six months shed little light on the overall, long-term safety of the product for approval.

As the Opinion acknowledges, no specific studies have looked at the effects of consuming trans-SDA.

No data has been provided for food safety in children.

No evidence has been provided to give confidence that there are no long-term adverse effects in humans.

The toxicology assessment concentrates on the newly expressed proteins *Primula juliae* $\Delta 6$ desaturase (Pj $\Delta 6$ D) and *Neurospora crassa* $\Delta 15$ desaturase (Nc $\Delta 15$ D) and on the four fatty acids stearidonic acid (C18:4; SDA), γ -linolenic acid (C18:3; GLA), 9c,12c,15t trans-ALA (C18:3) and 6c,9c,12c,15t trans-SDA (C18:4) produced in seeds of soybean MON 87769 normally not present at detectable levels in non-GM soybean seeds.

Although requested, the applicant was unable to provide 28-day repeated dose studies on the newly expressed proteins, owing to technical difficulties in obtaining purified proteins in an amount suitable for toxicological studies. EFSA concludes that "*that there are no reasons to suppose that these specific desaturases would introduce safety concerns*". But this ignores the vast literature on fatty acids which shows complex and possible unintended effects (such as possible increased risk of cancer). These effects cannot be studied without large-scale human trials. The evidence provided is therefore totally inadequate to assess the risks of making these substantive changes to the human diet.

Allergenicity

One portion of the query protein aligned with a sequence of nine consecutive serine residues in *Triticum aestivum* serine carboxypeptidase. Allergenicity should therefore have been further investigated. The reported study size (16 individuals clinically documented to be allergic to soybean and six non-allergic individuals) is inadequate.

Nutritional assessment

Nutritional and food safety assessment are linked (see comments above). Individuals will vary considerably in their dietary intakes and the impacts of altering fatty acid profiles are poorly understood. The assessment focuses on SDA as the most significant modification in MON 87769 soybean oil, and on the consequences of the reduction in the level of the essential fatty acid linoleic acid, but there are many complex nutritional changes in the soybean that are ignored. The applicant estimates SDA intake but cannot complete the analysis even for this single change because there is no dietary reference value for SDA and limited evidence in the literature of its impacts on health. Impacts of altering levels of DHA and EPA are also poorly understood (see above). Estimates of conversion rates are in any case highly speculative.

No data for children has been provided. No data has been provided for different age groups, needed to assess risk to specific subgroups of consumers. Some such information (including intakes for toddlers, children, teenagers, adults and the elderly, before and after the substitution of foods containing the GM soybean oil) was provided in the EFSA's statement complimenting its scientific opinion for Pioneer's GM soybean 305423. The lack of any such data here raises questions about

consistency and the need for a level playing field. The applicant should be required to supply this information as it is essential to underpin any nutritional assessment.

EFSA Guidance and Codex Guidelines require population subgroups to be considered in the nutritional assessment. As well as categories by age, this should include other subgroups whose nutrient requirements may be different from the general population. Again, this work has been totally omitted. It is impossible to completely fill this gap in these short comments, however there are a number of monogenic disorders, for example in the category of Fatty Acid Metabolism Disorders (MCAD, LCAD and SCAD deficiencies) in which medium-chain triglycerides (MCTs) can't be broken down and linoleic acid deficiency may occur (Acosta PB: http://www.fodsupport.org/pdf/Nutrition_and_Fatty_Oxidation_Defects.pdf) and others, such as Waldmann's disease, which require MCT supplementation (Vignes S, Bellanger J. Primary intestinal lymphangiectasia (Waldmann's disease). Orphanet Journal of Rare Diseases. 2008;3(1):5. doi:10.1186/1750-1172-3-5). Patients with Refsum's Disease are advised to eat soya products based on the level of phytanic acid they contain (<http://www.refsumdisease.org/patients/dietwhichfoods.shtml>) and patients with propionic academia are also unable to process certain lipids (<http://ghr.nlm.nih.gov/condition/propionic-acidemia>). The implications of altering fatty acid profiles in soybean oil should have been considered for such groups.

The EFSA GMO Panel concludes that the estimated decrease in the LA intake of adults is not of safety concern, despite the lack of an estimate for young children or for potentially vulnerable population subgroups. Linoleic acid contributes to the maintenance of normal blood cholesterol levels so it is surprising that no further data was required. Some studies suggest beneficial effects from high intake of linolenic acid (which is reduced in soybean MON87769) (e.g. Djoussé L, Hunt SC, Arnett DK, Province MA, Eckfeldt JH, Ellison RC. Dietary linolenic acid is inversely associated with plasma triacylglycerol: the National Heart, Lung, and Blood Institute Family Heart Study. Am J Clin Nutr. 2003;78(6):1098–1102). Why was the nutritional impact of this change not properly considered?

The Opinion relies heavily on the fact that EFSA (2010b) has set an adequate intake (AI) level of 250 mg EPA + DHA/day for adults, based on considerations of cardiovascular health. This is inadequate for a number of reasons including: (i) the report is out of date and more recent studies must be included (e.g. as cited above); (ii) it does not consider population subgroups who may be particularly affected by changes in the fatty acid profile of their food; (iii) it requires an extrapolation, based on limited data, of the impacts of the product on EPA+DHA and ignores other nutritional changes (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.

No systematic review has been provided of the nutritional evidence from the literature.

GeneWatch UK considers the lack of any proper nutritional assessment to be a serious omission. 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.

Others

The Opinion states (page 6): *“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”*.

However, it is difficult to understand how the product can be approved for use on the EU market unless all its potential uses on the market are considered.

The scope defined by the applicant (page 5): *“includes all food and feed products containing, consisting or produced from soybean MON 87769 including products from inbreds and hybrids obtained by conventional breeding of this soybean product. The application also covers the import and industrial processing of soybean MON 87769 for **all potential uses** as any other soybean”* [emphasis added].

It is therefore difficult to understand why the safety assessment does not cover all possible markets within the scope.

Based on the data provided, the EFSA GMO Panel concludes that feeding of full-fat soybean MON 87769 or inclusion of the oil derived from MON 87769 could alter the lipid content of animal tissues. However, the Panel did not consider the nutritional impact by consuming products of animal origin derived from animals fed whole fat MON 87769 or its oil on consumers. This approach is not compatible with the stated scope of the application.

Nutrient (and anti-nutrient) composition should be required for meat, milk and eggs from animals fed on soybean MON87769, since such uses can be anticipated. 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). Additional data should be requested from the application to cover these scenarios, to underpin a revised nutritional assessment.

Similarly, the possible use of the oil in supplements needs to be part of the assessment.

3. Environmental risk assessment

4. Conclusions and recommendations

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

5. Others

6. Labelling proposal

The applicant proposed that food and feed products within the scope of the application should be labelled as —genetically modified soybean containing SDA omega-3 oil or —contains genetically modified soybean containing SDA omega-3 oil.

This is factually incorrect since there is no omega-3 oil produced by the soybean. The label should describe the altered composition in full, including all the new fatty acids (stearidonic acid (SDA), also known as octadecatetraenoic acid; alpha-linolenic acid; and two trans-fatty acids, 9c,12c,15t trans-ALA (18:3) and 6c,9c,12c,15t trans-SDA (C18:4)) and the reduction in linoleic acid (LA). It is particularly important that consumers are warned about low linoleic acid, given the potentially adverse effects of this nutritional change.

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 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, or to ensure they are consuming adequate levels of others (such as LA).

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 MON87769 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.

