GeneWatch UK comments on Overall opinion on application for authorisation of genetically modified soybean 305423 for food and feed uses submitted under Regulation (EC) No 1829/2003 by Pioneer (EFSA-GMO-NL-2007-45)

1. **Sender: GeneWatch UK**
2. **Do you agree to publication of your comments (yes)**
3. **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)

A single field trial (at 10 US sites only in a single year) is 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, Pioneer's soybean 305423 application contains worrying signs of unintended effects on nutrient composition (as noted above) which should warrant more data being supplied not less. Statistically significant differences in 51 parameters, including fibre, minerals and phytoestrogens not intended to be altered by the modification, merit considerably more detailed investigations. Further data from other sites (including South America) and different years should be requested from the applicant.

b. Food Safety Assessment:

There is an extremely worry and puzzling lack of data provided here on the effects of processing on the nutrient profile (especially the fatty acid profile) of the soybeans. Processing is known to significantly alter nutrient levels and some such data was required for the MON87705 application (EFSA,2012) it is therefore hard to understand why it was not requested here. This data is essential before any meaningful nutritional assessment can be conducted. It must include information on nutrient and anti-nutrient levels and on bioavailability and bioefficacy taking onto account the potential influences of transport, storage and expected treatments of the food.

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. 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). Additional data should be requested from the application to cover these scenarios, to underpin a revised nutritional assessment.

Toxicology

The animal studies provided are inadequate to cover the required assessments. For example, in the rat studies reported, soybean 305423 was not treated with the intended herbicide. GeneWatch UK is not aware of any studies regarding nutrient composition and ALS-inhibiting herbicides, however it is well established in the case of the more commonly grown glyphosate-resistant GM crops that 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.

The feeding studies also omit any study of the effect of the altered nutrient profile on health: this is important because the purpose of the toxicological assessment is to demonstrate that the intended and unintended effects of the genetic modification have no adverse effects on human or animal health. Relevant questions and endpoints might be, for example, whether the altered fatty acid composition (increased omega-3 PUFAs and MUFAs) might increase the risk of breast or prostate cancer (see comments on nutritional assessment below). The applicant should be required to submit a detailed risk assessment on these aspects.

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. As noted above, nutrient profiles for meat and milk should also be provided.

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 serious omission has perhaps occurred because there are no nutritionists on the GMO Panel (although one expert from the NDA has acted as a hearing expert) which means the panel lacks the relevant expertise to conduct a nutritional assessment.

GeneWatch UK considers the lack of any proper nutritional assessment to be the most 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.

Serious limitations on compositional information (nutrient profiles) have been noted above. In addition, no data has been provided for the 97.5th percentile intakes, needed to assess risk to more highly exposed consumers. Some such information was provided in the EFSA's statement complimenting its scientific opinion for MON87705, again raising questions about the lack of a level playing field.

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.

It is startling that there are no references to any of the extensive literature on nutrition in the scientific assessment. The starting point of any nutritional assessment must be a comprehensive literature review. Since nutrition studies rarely provide definitive conclusions, there is a need to weigh up the evidence taking into account the need for a precautionary approach. This is because new studies can support or reverse previously held views and the ability of consumers to avoid products based on new evidence (or retailers to withdraw them or manufacturers to change formulations) is much lower in the case of an oil likely to be used in multiple products than it is for supplements (which people can simply choose not to buy). The applicant should be required to provide a systematic review of studies published in the scientific literature and to submit new studies without delay should they arise during the course of consideration of the application. Without such a review hazard identification and hazard characterisation are likely to be incomplete and risk characterisation cannot be completed.

It is impossible to fill the important gap left by the lack of nutritional assessment in these short comments, but examples of studies that should be considered include:

- Studies suggesting a link between omega-3 fatty acids and prostrate cancer (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).
- Studies suggesting a link between oleic acid/MUFAs and breast cancer (Chajès V, Thiébaut ACM, Rotival M, et al. Association between Serum trans-Monounsaturated Fatty Acids and Breast Cancer Risk in the E3N-EPIC Study. Am J Epidemiol. 2008;167(11):1312–1320; Saadatian-Elahi M, Norat T, Goudable J, Riboli E. Biomarkers of dietary fatty acid intake and the risk of breast cancer: A meta-analysis. International Journal of Cancer. 2004;111(4):584–591).
- Studies suggesting a link between MUFAs and poor memory function (Gibson EL, Barr S, Jeanes YM. Habitual fat intake predicts memory function in younger women. Front Hum Neurosci. 2013;7:838).
- Studies suggesting beneficial effects from high intake of linolenic acid (which is reduced in soybean 305423) (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).

The nutritional assessment must also consider the outcomes of animal feeding studies but this is impossible without further information from the applicant because: (i) (as noted above) the rat feeding study supplied did not include soybean oil from soybean305423 treated with the intended herbicide (and this is important because it is likely to change nutrient levels); (ii) foods utilising the GMO (as opposed to the GMO itself) were not included in any animal feeding study so no data of relevance to human consumption of these foods was obtained; (iii) appropriate endpoints were not considered. Further feeding studies are therefore necessary to consider the nutritional impacts of all the food products intended for human consumption that are included within the scope of the application.

Although animal feeding studies are required as a first step, credible evidence of relative benefits and harms associated with the substantially altered fatty acid profile and other nutrient changes in soybean 305423 in terms of endpoints such as cardiovascular or cancer risk may only be obtained by conducting large-scale long-term clinical trials in humans. Relevant studies of this type should therefore also be provided.

These studies should be considered in the context of the latest evidence which suggests no consensus on the benefits of MUFAs for cardiovascular disease (Schwingshackl L, Hoffmann G. Monounsaturated Fatty Acids and Risk of Cardiovascular Disease: Synopsis of the Evidence Available from Systematic Reviews and Meta-Analyses. Nutrients. 2012;4(12):1989–2007) and a Cochrane Review which identifies possible benefits of dietary fat modification in terms of cardiovascular events but no overall confirmed effect on mortality (Hooper L, Summerbell CD, Thompson R, et al. Reduced or modified dietary fat for preventing cardiovascular disease. In: The Cochrane Collaboration, Hooper L, eds. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2011. Available at: http://doi.wiley.com/10.1002/14651858.CD002137.pub2. Accessed January 15, 2014). Further, it should be borne in mind that any benefits that might exist could be achieved my means other than introducing soybean oil with a substantially altered and untested fatty acid profile into the food chain.

There are many gaps in the literature, leading to a lack of understanding, for example, of the implications of altering fatty acid profiles in foods for babies and young children. Although data has been supplied on estimated daily intakes for toddlers, children, teenagers, adults and the elderly, it is unclear how this relates to the (missing) compositional analysis of different foodstuffs such as margerines and snacks and no data on bioavailability or the nutritional status of different subgroups likely to consume the food has been provided. This data should be requested from the applicant.

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.

Finally, as noted above, the potential for soybean 305423 to be fed to animals as a supplement (i.e. as oil or seeds, not solely as defatted meal) and alter the nutrient profiles of meat, milk or eggs has yet to be considered. Additional data is required from the applicant to consider this scenario.

In GeneWatch's view the existing literature suggests that it is extremely questionable whether soybean 305423 should be allowed on the market, particularly when the options for recall or consumer avoidance may be difficult (see comments on labelling below).

Others

Since the application covers the authorisation of the GMO and its use in assorted foods, consumption of all of these foods must be monitored as part of the post-market monitoring. Effects on health should also be monitored but it is impossible to specify monitoring requirements in the absence of a nutritional assessment (as noted above).

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 labelling proposal "genetically modified soybean with altered fatty acid profile" is 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 DP-305423-1). 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 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.