Soybean 305423 x 40-3-2

Organisation: Monsanto
Country: Sweden
Type: Consultant

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a. Assessment:
b. Food Safety Assessment:
Toxicology

Too much

Allergenicity

Very high

Nutritional assessment

Non what so ever.

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3. Environmental risk assessment

Soy bean cause deforestation. 91 % of amazonas does not ecist anymore.

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4. Conclusions and recommendations

Stop gmo and monsanto

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6. Labelling proposal

Label as dangerous

Organisation: EFSA
Country: Italy
Type: Scientific Institution

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a. Assessment:
5. Others

Technical test

Organisation: ANFNC (Feed Manufactures Association)
Country: Romania
Type: Association

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a. Assessment:
Molecular characterisation

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

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b. Food Safety Assessment:
Toxicology

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Allergenicity

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Nutritional assessment

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Others

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3. Environmental risk assessment

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4. Conclusions and recommendations

The hi-oleic soybean varieties have important health benefits for consumers – and this should be particularly the interest in Europe, where diet patterns still expose millions of people at risk from high trans fatty acid content in food.

Beyond this, however, the feed manufacturers in Romania and all over Europe consider of utmost importance to have the Commission act timely on putting on the right track the new GM products that received EFSA's positive safety assessment opinion. Just like the rest of EU, Romania, needs to import around to 70% of its total protein feed requirements, mainly from American countries. Therefore, and any postponement in approving these products already grown in other parts of the world may cause costly trade disruptions, hurting the entire chain: producers, livestock farmers, processors (including feed industry), traders, and, ultimately, consumers.

5. Others

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Organisation: GeneWatch UK
Country: United Kingdom
Type: Non Profit Organisation

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.

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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., & Surgan, 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 syngergistic 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.

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.

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.

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.

Organisation: Institute of Agricultural Economics -Romanian Academy
Country: Romania
Type: Scientific Institution

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a. Assessment:
b. Food Safety Assessment:
Toxicology

All the potential risk were take in consideration by EFSA

Allergenicity

All the potential risk were take in consideration by EFSA

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3. Environmental risk assessment

All the potential risk were take in consideration by EFSA

4. Conclusions and recommendations

Healthy populations have varied levels of dietary fat intake and minimal intake of industrial-produced trans fats, low quantities of saturated fatty acids and relatively high quantities of monounsaturated fatty acids. A major issue in Europe are the heart coronary diseases, because of both diet patterns and lifestyle. Dietary guidance across the majority of European countries includes advice to reduce saturated and trans fatty acids. The high oleic soybean development is an important achievement in this regard. Plenish high oleic soybean oil is high in heart-healthy monounsaturated fat (more than 75 percent oleic content, or Omega-9). Beyond the health benefits, an important economic advantage its low production cost comparative to raw material for similar oils (olive oil) that provide cardiovascular protection.

5. Others

Given the current levels of global trade in agricultural commodities, and being well known that these products are already grown in other parts of the world, in the absence of a technical solution related to AP/LLP in food, the European market is faced with an increased risk of non compliance and of legal uncertainty. The foreseeable trade disruptions will not only affect various European industry sectors, but will ultimately affect consumers. The GM products assessed by EFSA to pose no risk to consumers should be approved timely by the EC.

Organisation: Agrobiotechrom
Country: Romania
Type: Individual

a. Assessment:
Molecular characterisation

Assessed by EFSA.

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

Assessed by EFSA.

b. Food Safety Assessment:
Toxicology

Assessd by EFSA.

Allergenicity

Assessed by EFSA.

Nutritional assessment

Assessed by EFSA.

Others

No.

3. Environmental risk assessment

Soybean is a very sensitive crop. Soybean seeds rarely survive during the winter and if they survive can be easily controled. Also soybean is a self poilinated crop and do not spread pollen into environmen.

4. Conclusions and recommendations

I agree EFSA conclusion.

5. Others

EU member state import more than 30 million tones of soyben an soy meal from US, Brasil

and Argentine. Approval delay of the introduction of this variety in consomtion disrupt trade flows supply for soybeans and soybean meal needed in animal feed.

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6. Labelling proposal

I agree with EFSA recomandations.

Organisation: individual
Country: Sweden
Type: Others...

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a. Assessment:
Molecular characterisation

Soybean 305423 x 40-3-2: The cp4 epsps transgene, leading to tolerance to glyphosate, was inherited from soybean line GTS 40-3-2

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b. Food Safety Assessment:
Toxicology

The safety of glyphosate i still debated:

A. Safety is highly questioned according to a consensus statement by Myers et al, published 17th of Februari 2016 in Environmental Health:
https://ehjournal.biomedcentral.com/articles/10.1186/s12940-016-0117-0

B. "Conflict of interests" is alleged in the latest classification of Glyphosate by the UN as "probably not carcinogenic to humans":
https://www.theguardian.com/environment/2016/may/17/unwho-panel-in-conflict-of-interest-row-over-glyphosates-cancer-risk

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Others

3. Environmental risk assessment

See point B, under Food Safety Assessment.

4. Conclusions and recommendations

1. Consider the consensus statement by Myers et al:
https://ehjournal.biomedcentral.com/articles/10.1186/s12940-016-0117-0

2. Investigate alleged conflicts or interest in the UN Panel, stating Glyphosate as "probably not carcinogenic to humans":
https://www.theguardian.com/environment/2016/may/17/unwho-panel-in-conflict-of-interest-row-over-glyphosates-cancer-risk

3. Alongside the statement on Glyphosate from FAO/WHO, after the meeting 9-13 May 2016 (http://www.who.int/foodsafety/jmprsummary2016.pdf), a Q&A document on Glyphosate from IATRC was issued (https://www.iarc.fr/en/media-centre/iarcnews/pdf/Q&A_Glyphosate.pdf). According to Reuters, WHO explained their view upon the contradictory results of FAO/WHO and IARC as follows: "IARC reviews published studies to identify potential cancer hazards," the WHO said. "It does not estimate the level of risk to the population associated with exposure to the hazard." (http://www.reuters.com/article/us-health-who-glyphosate-idUSKCN0Y71HR)

NOTE: The main role for EFSA must be to protect the EU citizens from a potential cancer hazards! Not to estimate the level of an expressed risk!

5. Others

The 29th of July 2016, several plaintiffs turned to the U.S. Judicical Panel on Multidistrict Litigation (JPML) in order to coordinate pending lawsuits against Monsanto/Glyphosate causing cancer. Doing so they cited more than 20 similar cases pending:
http://www.aboutlawsuits.com/wp-content/uploads/2016-07-29-Response.pdf

With little surprise it took Monsanto only 2 weeks to oppose:
http://www.aboutlawsuits.com/wp-content/uploads/2016-08-18-Opposition.pdf

The outcome of these lawsuits could change the safety claims of Glyphosate in the years to come.

Organisation: Testbiotech
Country: Germany
Type: Non Profit Organisation

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a. Assessment:
Molecular characterisation

Both parental soybeans were produced by particle bombardment. This method is known to have a major impact on plant DNA (see, for example, Makarevitch et al., 2003).

In soybean 305423, molecular characterisation revealed multiple rearrangements, and several complete and truncated copies of gene constructs were detected. Soybean 305423, for example, contains in total, eight copies of the KTi3 promoter, seven copies of the gm-fad2-1 gene fragment and five copies of the KTi3 terminator. Gene products such as RNA produced from these additional and unintended copies can render various biological effects. One unintended effect - in regard to the KTi3 gene - is evident from the data provided on the plant's composition, which show a reduction in the concentration of the plant´s own trypsin inhibitor protein. It is likely that this effect results from RNAi. The molecular characterisation has also revealed that one of the investigated plants showed signs of genetic instability.

In soybean 40-3-2, Rang et al (2005) showed occurrence of unintended open reading frames, due to the non-functioning of the nos-stop codon, causing the occurrence of additional RNA in the plants. Even though no fusion proteins were identified the plants produce additional RNA that needs further assessment. For example, if small double stranded RNA is produced it could be transmitted as a biologically active compound at the stage of consumption. However, no detailed investigations were performed to assess these unintended gene products in detail.

In general, beyond that, RNAi effects are highly relevant for the risk assessment of these genetically engineered soybeans: RNAi is used to achieve the intended changes in the oil quality of soybean 305423. Therefore, the assessment of the biological effects of intended and unintended miRNA produced in the plants should have been a priority. However, no investigations were requested to assess the newly produced miRNA in detail.

In 2012, it was reported for the first time that miRNA produced by plants can enter the blood of mammals (including humans) at the stage of consumption (Zhang et al, 2012). These findings were called into question by several experts. However, looking at more recent publications one has to assume that the plant miRNA can indeed enter the blood, organs and urine of mammals after ingestion (Beatty et al., 2015; Yang et al., 2015; Liang et al., 2015; Hirschi et al, 2015). Certainly, the amount being taken up and the biological impact depend on factors that need further research.

This uptake of small RNAs via ingestion is relevant for risk assessment. There is evidence that small RNAs taken up from the intestine do indeed interfere with gene regulation in humans and animals. For example, it was found that miRNA transferred via milk shows biological activity (Baier et al., 2014; see also: Lukasik & Zielenkiewicz, 2014). Small RNAs produced by plants are able to interfere with the immune system in humans and animals (Zhou et al., 2015; Cavalieri et al., 2015).

Therefore, EFSA should have requested data on the emergence of new variations, combinations and concentrations of small, biologically active RNA in the parental plants as well as in the stacked event.

Furthermore, both the expression of the enzymes that confer herbicide resistance and the concentration of small biologically active RNA molecules should have been tested under a wide range of defined environmental conditions, taking into account stressful conditions that, for example, emerge under ongoing climate change. It is known that under stress conditions, genetically engineered plants can show reactions that are not obvious under normal agricultural conditions, and these can be very different from those of conventionally bred plants (see, for example, Gertz et al., 1999). Environmental stress can also cause unexpected patterns of expression of the newly introduced DNA (Trtikova et al., 2015).

Finally, since the KTi3 gene inserted into the plants unintentionally renders biological effects (lower content of the trypsin inhibitor protein in the plants), the expression rate of the additional KTi3 gene and its specific gene products should also have been investigated in detail.

To summarise, the inserted DNA, its truncated sequences, rearrangements and open reading frames can interfere with gene regulation in the plants and cause a broad range of unintended effects. Apparently such effects occur, since the plants show a lower content in trypsin inhibitor and a reduction in odd chain fatty acids. Metabolic and genomic screening would be required to assess the real magnitude of these effects and to exclude other effects. In these investigations, the plants should also be subjected to defined environmental stress factors. But no such investigations were requested by EFSA.

Baier, S.R., Nguyen, C., Xie F., Wood J.R. Zempleni J. (2014) MicroRNAs Are Absorbed in Biologically Meaningful Amounts from Nutritionally Relevant Doses of Cow Milk and Affect Gene Expression in Peripheral Blood Mononuclear Cells, HEK-293 Kidney Cell Cultures, and Mouse Livers 1–3 , The Journal of Nutrition, p.p. 1495-1450

Beatty, M., Guduric-Fuchs, J., Brown, E., Bridgett, S., Chakravarthy, U., Hogg, R.E., et al. (2014) Small RNAs from plants, bacteria and fungi within the order hypocreales are ubiquitous in human. Plasma, 15: 1–12. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4230795/

Cavalieri D., Rizzetto L., Tocci N., Rivero D., Asquini E., Si-Ammour A., Bonechi E., Ballerini C., Viola R. (2016) Plant microRNAs as novel immunomodulatory agents , Scientific Reports, 6:25761, DOI: 10.1038/srep25761, www.nature.com/scientificreports/

Gertz, J.M., Vencill, W.K., Hill, N.S. (1999) Tolerance of Transgenic Soybean (Glycine max) to Heat Stress, British Crop Protection Conference – Weeds, 15-19 Nov 1999,. Brighton: 835-840.

Hirschi, K. D., Pruss, G. J. & Vance, V. (2015) Dietary delivery: a new avenue for microRNA therapeutics? Trends Biotechnol. 33, 431–432, doi: 10.1016/j.tibtech.2015.06.003.

Liang, H., Zhang, S., Fu, Z., Wang, Y., Wang, N., Liu, Y., ... & Chen, X. (2015) Effective detection and quantification of dietetically absorbed plant microRNAs in human plasma. The

Journal of nutritional biochemistry, 26(5): 505-512.
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Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)

Despite field trials only being conducted in the USA and Canada and only for one year, significant differences were found for several compounds and agronomic characteristics. These differences should have been investigated over more than one year and under a broad range of environmental conditions, including defined biotic and abiotic stressors.

Some of the significant changes observed, such as lower content of trypsin inhibitor and odd chain fatty acids, are apparently caused by unintended effects due to the insertion of the additional DNA.

As a result, the soybean shows a wide range of intended and unintended changes in its composition and agronomic characteristics, and therefore has to be regarded as substantially different from its conventional counterparts used as comparators. As the EFSA Guidance Document from 2006 shows, this requires more in-depth investigation of the whole food and

feed, regardless of whether the observed effects are known to be detrimental to human or animal health: “If the composition of the GM plant is modified substantially, or if there are any indications for the potential occurrence of unintended effects, based on the preceding molecular, compositional or phenotypic analysis, not only new constituents, but also the whole GM food/feed should be tested. In such a case, the testing programme should include at least a 90-day toxicity study in rodents”.

However, EFSA did not request any further investigation such as testing of the whole food and feed.

EFSA (2006) Guidance document for the risk assessment of genetically modified plants and derived food and feed by the Scientific Panel on Genetically Modified Organisms (GMO), Available online: www.efsa.europa.eu

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b. Food Safety Assessment:
Toxicology

The applicant provided a 90-day feeding study of insufficient quality and so it was rejected by EFSA. However, EFSA should have consequently requested a new study, due to the many intended and unintended effects observed in the composition of the plants.

It should also be taken into account that the feeding studies with the parental plant 305423 suffer from major deficiencies. Furthermore, according to Magaña-Gómez et al. (2009), a number of the studies with soybean 40-3-2 revealed signs of possible health effects.

Thus, investigations with the whole food and feed are definitely needed for the risk assessment of the combination of the two soybeans. This was also noted by experts from Member States (EFSA, 2016 b) such as the German authority, the Federal Office of Consumer Protection and Food Safety (BVL).

However, EFSA failed to ensure that necessary data were provided.

Also relevant in this context, but omitted in the risk assessment of the GMO Panel, is the potential toxicity caused by the residues from spraying with the complementary herbicides. Due to the specific agricultural practices that go along with the cultivation of these herbicide resistant plants, there are, for example, specific patterns of applications, exposure, occurrence of specific metabolites and emergence of combinatorial effects that require special attention. For example, commercial large-scale cultivation of these plants results in a strong selective pressure on weeds to develop resistance to these herbicides (Sammons & Gaines, 2014), this can lead to increasing amounts of sprayed herbicides and subsequently of residues in the harvest. Further, herbicide-tolerant plants are meant to survive the application of the complementary herbicide while most other plants will die after short time. Thus, for example, residues of glyphosate, its metabolites and additives to the formulated product might accumulate and interact in the plants. As a publication by Kleter et al. (2011) shows, using herbicides to spray genetically engineered herbicide-resistant plants does indeed lead to patterns of residues and exposure that are not taken into account in regular pesticide registration: “1. GM herbicide-resistant crops can change the way that herbicides can be used

on these crops, for example: (a) post-emergent over-the-top applications (i.e. on the crop itself) instead of directed sprays, avoiding herbicide contact with the crop; or (b) pre-emergent and pre-harvest applications made to the conventional crop and not, or in different quantities, to the GM crop. 2. The residue profile of the applied pesticide may have been altered on the basis of the nature of the modification. 3. The overall pattern of pesticides applied to the particular crop may have been altered, leading to different exposure to pesticide residues overall."

According to a reasoned legal opinion drawn up by Kraemer (2012), residues from spraying with complementary herbicides have to be taken into account in the risk assessment of genetically engineered plants from a regulatory point of view: "It is the objective of Directive 2001/18 to avoid any adverse effect of the genetically modified plant on human health. The provisions of the Directive on the environmental risk assessment are very broad and try to cover - in the abstract, it is true – all possible cases, where direct or indirect, immediate, delayed or unforeseen adverse effects might occur. Then, it is only logical that, when genetically modified plants which are tolerant to certain herbicides, are exposed to pesticide or herbicide treatment, the effects of such treatment on the plant – and later on human or animal health – must be examined during the environmental risk assessment."

Following on from this, that the applicants have to provide a comprehensive environmental risk assessment of the genetically engineered plants, which includes all and potential adverse effects on the environment as well as on human and animal health. This requirement includes long-term potential and accumulative effects and also all other harmful effects on human or animal health which are, in one way or another, related to the genetically modified plant, such as residues from spraying with complementary herbicides.

This is also in accordance with pesticide regulation, which requires specific risk assessment of imported plants if the usage of pesticides is different in the exporting countries compared to the one in the EU: Recital 26 of Regulation 396/2005 requires Maximum Residues Levels (MRLs) are set for food and feed produced outside the Community if produced by different agricultural practices as regards the use of plant protection products. Article 14 of Regulation 396/2005 requires that the presence of pesticide residues arising from sources other than current plant protection uses and their known cumulative and synergistic effects are determined. Further, Article 29 of Regulation 1107/2009 states that active substances and synergists have to be approved, and the maximum residue levels for each specific agricultural products have to be determined.

In any case, both the EU pesticide regulation and the GMO regulation require a high level of protection for health and the environment. Thus, in regard to herbicide-resistant plants, specific assessment of residues from spraying with complementary herbicides must be considered to be a prerequisite for granting authorisation. In addition, cumulative effects have to be investigated if a plants contains or produces other compounds of potential toxicity.

A basic prerequisite for risk assessment in this context is the availability of valid and reliable data on residue loads from spraying with herbicides. This is especially relevant in the case of glyphosate: A study published in 2015 (IARC) found that glyphosate is probably carcinogenic. While carcinogenicity of the active ingredient remains a matter of debate (EFSA 2015 a), there is a scientific consensus that additives and their mixtures used in commercial formulations for spraying glyphosate can show a much higher toxicity than the active ingredient alone (Mesnage et al., 2015). The amount of these residues depends on the

specific agronomic management used in the cultivation of the herbicide resistant plants. Data from some publications (Bøhn et al., 2014, Cuhra, 2015) show, a considerable amount of residues from spraying can be expected in genetically engineered soybeans resistant to glyphosate formulations. In general, the level of residues is likely to increase due to increasing problems with herbicide resistant weeds (Benbrook, 2016)

However, as the EFSA Pesticide Panel stated (EFSA 2015 b), safety of residues from spraying glyphosate formulations could not be concluded on the data provided so far. Thus, EFSA was unable to deliver a conclusive risk assessment on the actual risks of residues from spraying with glyphosate and the various glyphosate formulations.

Furthermore, there is no comprehensive risk assessment of residues from spraying ALS inhibitors as complementary herbicides on genetically engineered soybeans. On the opposite, major data gaps were identified by the Pesticide Panel of EFSA (EFSA, 2015c) in the case of thifensulfuron, which is one of the active ingredients that act as an ALS inhibitor: “Data gaps were identified in the residue section. Pending the ability of a sufficient evaluation of consumer exposure and/or further information on the toxicological profile for specific plant and livestock metabolites, the consumer risk assessment can not be finalised for the representative uses." and “In the area of mammalian toxicology and non-dietary exposure, data gaps were identified to define the toxicological profile of some metabolites and impurities. The equivalence of the different sources produced by Cheminova and Rotam to the agreed technical specification by DuPont (that was supported by the toxicological studies) should be re-assessed leading to a data gap. The potential endocrine disruption of thifensulfuron-methyl was identified as an issue that could not be finalised and a critical area of concern.”

As a result, risk assessment of the genetically engineered soybeans cannot be concluded. In this context, EFSA´s risk assessment omitted further relevant health risks: There is a considerable amount of literature indicating that glyphosate formulations can act as so-called endocrine disruptors (see, for example, Thongprakaisang et al., 2013; Caglar and Kolankaya, 2008; de Liz Oliveira Cavalli et al., 2013; Omran et al., 2013). Since soybeans also produce a number of plant estrogens (de Lemos, 2001), there might be some synergistic or additive interaction with the residues from spraying with glyphosate formulations. However, the impact of the soybeans on the hormone system of mammals was not investigated.

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Allergenicity

It is known that toxicants, if applied together with the allergens, can have adjuvant effects, triggering a stronger immune reaction to the proteins. This is a specific risk that needs to be addressed in the context of residues from spraying with the complementary herbicides.

Furthermore, soybeans are known to have a substantial variation in their natural concentrations, depending on specific varieties and on interaction with the environment. The applicant failed to show that the level of endogenic allergens in specific varieties and/ or under specific environmental conditions is not increased. For this purpose, further crossing with other varieties should have been performed as well as subjecting the soybeans to suitable tests including biotic and abiotic stressors.

The applicant provided data on testing with blood samples stemming from a small group of people known to be sensitive to soybean allergens to find out if they had a changed reaction to the genetically engineered soybeans. However, the number of samples used for testing was too small to get reliable results. Furthermore, no analysis was undertaken of the risks for individuals with an impaired immune system such as the elderly or infants, as requested by the EFSA guidance (EFSA, 2010).

EFSA (2010) EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010; 8(7):1700. [168 pp.] doi:10.2903/j.efsa.2010.1700. Available online: www.efsa.europa.eu

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Nutritional assessment

In the process of risk assessment, potential hazards need to be identified first, before the level of exposure is taken into account. However, EFSA appears to be setting the decisive steps in risk assessment aside: Based on data for average exposure of consumers, it is concluded that no further risk assessment is needed.

The average exposure is far from being reliable: Only the anticipated average but not maximum intake of soybean food in Europe was estimated. In reality, habits regarding the consumption of soybean products can vary greatly over time, in different regions, subpopulations and individuals.

Thus, the applicant has to show that all relevant food products are safe, for all kind of diets as well as accumulated and long-term effects. However, there are no data on the safety of products that are processed, such as soybean milk and baby food. Without such data, no conclusion can be drawn upon food safety. 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, including data on secondary products such as soy lecithin.

Since the soybeans are intentionally and unintentionally are changed in their oil composition, there are many open questions regarding the potential health effects of the products derived from the soybeans. For example, a higher concentration of MUFA as well as a lower content in odd chain fatty acids are under discussion regarding negative health effects (Jenkins, et al., 2015, Chua et al., 2013).

Long-term feeding studies including various concentrations of the relevant fatty acids would be needed, to conclude on the safety of the products derived from the soybeans. However, from an ethical point of view there are considerable doubts about whether the potential benefits of these soybeans would justify such trials.

Chua, M.E., Sio, M.C.D, Sorongon, M.C., Morales, M.L. (2013) The relevance of serum levels of long chain omega-3 polyunsaturated fatty acids and prostate cancer risk: A meta-analysis. Can Urol Assoc J. 7(5-6):E333–343.

Jenkins B., West J.A., Koulman A. (2015) A Review of Odd-Chain Fatty Acid Metabolism and the Role of Pentadecanoic Acid (C15:0) and Heptadecanoic Acid (C17:0) in Health and Disease , Molecules 20, 2425-2444

Others

As a legal dossier compiled by Professor Ludwig Kraemer (Kraemer, 2012) shows, EU regulations require the monitoring of effects on health at the stage of consumption in cases where there are uncertainties. Thus, for example, there must be a requirement for the monitoring of health effects that takes residues from spraying with herbicides into account.

In this case, case specific monitoring would be needed to investigate negative health impacts from residues of spraying as well as effects stemming from the intended and unintended changes in the plants´ composition. Further, any spillage of the kernels has to be closely monitored, since the data on agronomic characteristics show significant changes in the performance of the plants.

Kraemer, L. (2012) The consumption of genetically modified plants and the potential presence of herbicide residues, legal dossier compiled on behalf of Testbiotech, http://www.testbiotech.de/sites/default/files/Legal_Dossier_Kraemer_Pesticide_RA_PMP.pdf

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4. Conclusions and recommendations

Based on the data presented and assessed, the risk assessment cannot be concluded. Consequently, the application should be rejected.