RAGES
RISK ASSESSMENT OF GENETICALLY ENGINEERED ORGANISMS IN THE EU AND SWITZERLAND

Health risk assessment of genetically engineered nutritionally-altered GM crops

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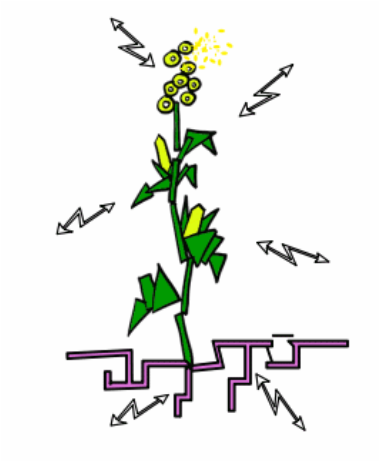

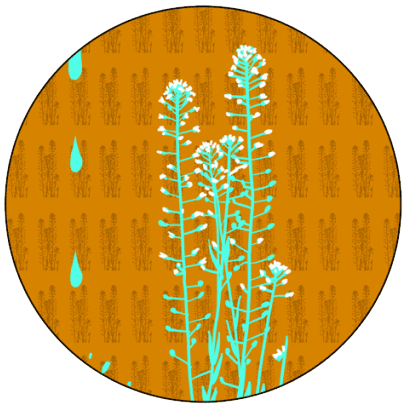

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Content

Summary

Three GM crops with altered oil content have already been approved for import to the EU and use in food and feed. In future, other nutritionally altered GM crops – for example, with altered vitamin or mineral content – might be proposed for import or for cultivation, or might pose risks associated with the accidental spillage of crops granted authorisation for feed and food uses only.

Nutritionally altered crops pose challenges for risk assessment. Nutritional changes are complex and not limited to a single nutrient and their impacts may vary with dose and also depend on the receiving population, which will include vulnerable persons. Due to this complexity, risk assessment and labelling are both challenging. Nutritionally altered GM crops have been approved for use as food and feed within the EU without specific guidance for their risk assessment. This means that many important issues have not been considered adequately. Issues include:

- The GM traits all affect multiple nutrients and the overall effect on health is poorly understood: health claims (of benefits) are not substantiated;
- Because, unlike previous GM crops, nutritionally-altered GM crops are engineered to produce molecules that are biologically active in humans, there is an increase in the risk of adverse medical effects either from over exposure to the intended product or from unintended by-products whose hazard to health is unknown;
- Gene-environment interactions will affect nutrient expression and the field trials conducted are inadequate to characterise the resulting variability in nutrient levels;
- There has been no full nutritional/food safety analysis (instead the focus is on comparing the main altered nutrient with standard dietary recommendations);
- Potentially vulnerable subgroups need to be considered;
- Impacts of food processing and storage need to be considered for all food types;
- Use of the GM crop as feed can alter nutrient content of (unlabelled) meat and dairy products;
- Food labelling proposals are inadequate to provide sufficient information for consumers;
- Post-market monitoring is inadequate to identify adverse health effects.

Other nutritionally altered crops may in future contain altered levels of vitamins and minerals, which will pose additional challenges for risk assessment.

No applications for commercial cultivation of nutritionally-altered GM crops have been made to date in the EU or Switzerland. However, there are many examples of unintended effects described in the published literature. These include:

- Direct adverse effects on wildlife of consumption of altered nutrients;
- Complex ecological effects associated with introducing new or enhanced levels of nutrients into ecosystems;
- Increased attractiveness to pests and/or susceptibility to pathogens, associated with altering biochemical pathways in the plant;
- Adverse impacts on yield and agronomic properties.

In addition, contamination issues associated with nutrient-altered GM crops could be particularly significant: for example, with omega-3 altered GM oil seed rape being proposed for growing on an industrial scale for use as fish feed.

Introduction

This subreport considers the example of nutritionally-altered GM crops. It first discusses the example of three GM crops with altered oil content, which have already been approved for import to the EU and use in food and feed. Then, some potential future examples are considered of other nutritionally altered GM crops – for example, with altered vitamin or mineral content - and the challenges they pose for risk assessment are discussed. Finally, potential future cultivation of nutritionally altered GM crops is considered, including environmental risks that might occur from accidental spillage of crops granted authorisation for feed and food uses only.

In contrast to most GM crops currently on the market, nutritionally-altered GM crops are genetically engineered to produce small molecules that are biologically active in humans. This poses new challenges for regulators.

1. Oil-altered GM crops already approved for import for use in food and feed

The European Commission has issued market authorisations for the import and use in food and feed of several nutritionally-altered genetically modified (GM) soybeans with altered oil content. The market authorisations issued on 24th April 2015 (European Commission, 2015a, 2015b) were for:

- Monsanto's MON 87769 soybean: a stearidonic acid (SDA)-producing soybean;
- Monsanto's MON 87705 soybean: a glyphosate-tolerant low-linoleic, high-oleic soybean, known as Vistive Gold;
- Pioneer soybean 305423: a herbicide-tolerant (to (ALS)-inhibiting herbicides), high-oleic acid soybean, known as Plenish.

The European Food Safety Authority (EFSA)'s risk assessments for these crops were developed without first adopting guidance for the assessment of nutritionally-altered GM crops.
In contrast to most GM crops currently on the market, nutritionally-altered GM crops are genetically engineered to produce small molecules that are biologically active in humans. It is therefore particularly important to assess the impacts of these products on human health.

Environmental risks are not considered in this section, since the risk of accidental establishment of GM soybeans is low. However, such risks (which are considered in Section 3) may be important for future nutritionally-altered crops, such as GM oil seed rape, even if they are granted authorisations for food and feed use only.

In addition to the issues outlined below, which relate specifically to nutrient-altered GM crops, a number of other issues have not been fully considered in the risk assessments. In particular:

- MON 87705 and soybean 305423 are both genetically engineered to be tolerant to herbicides. As such, limitations in safety and nutritional testing described below should also have taken into account the presence of herbicide residues on these crops;
- In MON 87705, the genetic modification results in an inhibition of the expression of the FAD2-1A and FATB1-A genes by RNAi interference (RNAi). The use of RNA interference can give rise to unintended off-target effects (Heinemann et al., 2013; Lundgren and Duan, 2013) but this possibility has not been adequately investigated.

These issues are addressed in other subreports and are not considered further here, but it is important to note that they will add to the complexity of any risk assessment.

1.1 Lack of guidance for risk assessment of nutritionally altered crops

The three nutritionally-altered products which have been approved for import as food and feed are all soybeans which have been genetically engineered to express different fatty acids which alter the oil composition of the final crop. They are not regarded as "substantially equivalent" to existing conventional crops and therefore pose new challenges for regulators.

Substantial equivalence has no exact definition but it can be taken to mean that if a GM food can be characterized as substantially equivalent to its 'natural' antecedent, it can be assumed to pose no new health risks and hence to be acceptable for commercial use (Millstone et al., 1999). This concept is has been widely criticised and is discussed further in the subreport on Regulation. For this subreport, we simply note that there is a consensus that nutritionally-altered GM crops are not substantially equivalent to a conventional counterpart and therefore require some assessment of their altered properties.

EFSA is mandated to issue guidance on the manner in which it will assess applications for authorisations for genetically modified organisms (GMOs). In particular:

- Under Article 23(b) of Regulation 178/2002, one of EFSA's tasks is that it must *"promote and coordinate the development of uniform risk assessment methodologies in the fields falling within its mission"*;
- Under Articles 5(8) and 17(8) of Regulation 1829/2003 on genetically modified food and feed, EFSA *"shall publish detailed guidance to assist the applicant in the preparation and presentation of the application"*.

EFSA recognises the importance of developing methodologies in Section 5.2 of its Policy on Independence and Scientific Decision-Making Processes (*Policy on Independence and Scientific Decision-Making Processes of the European Food Safety Authority, 2011*). Guidance is also important to ensure a "level playing field" so that all products in a similar category (such as nutrient-altered GM crops) are subject to the same standards of regulatory oversight and assessment. Without Guidance, it is difficult to ensure that regulation is applied in a non-discriminatory manner. All GM nutritionally-enhanced plants are different and each will pose different risks, depending on their intended and unintended products and side-products.

However, despite highlighting the importance of developing standard methodologies to ensure impartiality and coherence of its outputs, EFSA has not completed work that it initiated in 2012 to develop the necessary guidance for the assessment of nutritionally altered crops. In Mandate Number M-2012-0084, EFSA has itself recognised the need to develop and detail a strategy for the safety and nutritional assessment of nutritionally altered GM crops, and its (former) Director commissioned the first step in this work. However, neither EFSA nor any other EU institution has taken subsequent steps to progress or finalise this work to create the necessary detailed strategy for the assessment of nutritionally-altered crops. EFSA now takes the view that its existing Guidance for the risk assessment of GM plants for food and feed uses is adequate and specific Guidance for nutritionally-altered crops is not needed.

However, under Mandate Number M-2012-0084, in June 2012, EFSA acknowledged that the process for evaluation of this new category of crops (including nutritionally enhanced foods with qualitative and quantitative changes in oils/lipids) required further study and development and

commissioned an expert report at a cost of 75,000 euros (Geslain-Lanéelle, 2012). EFSA's mandate letter cites EFSA's new (2011) Guidance for risk assessment of food and feed from genetically modified plants, which states (page 34):
"In cases where a comparative assessment is not applicable, a comprehensive food and feed safety and nutritional assessment of the GM plant and derived food and feed should be performed. This should include, among others, a detailed compositional analysis and specific toxicological/nutritional analyses, selected according to the agronomic and compositional properties of the food and feed under assessment. ***Further development and detailing of this strategy is needed.****"* [emphasis added]. The mandate letter states: *"In practical terms, such strategy for a comprehensive food/feed safety assessment, although being addressed in the guidance documents of the EFSA Panel on Genetically Modified Organisms,* ***has so far not been fully described.*** *For the assessment of applications of GM plants developed to express new traits the EFSA GMO Panel is currently receiving ad-hoc support from Nutrition (NUTRI) Unit and members of the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA).* ***The definition of clear strategies for the assessment of these applications is becoming a relevant issue for the GMO Panel.****"* [emphasis added].

In September 2013, EFSA published the expert report resulting from Mandate Number M-2012-0084, which considers in more detail the studies that would be necessary for regulatory approval of "novel" GM traits, including altered nutrient content (ADAS UK Ltd. & Rothamsted Research, 2013). In this report (page 3):
"A number of recommendations for further work are given, including the need for a wider review of risk assessment strategies to inform the approach to risk assessment for 'novel' traits, further work to develop guidance on post market monitoring, guidance on cases where field trial design for 'novel' traits may need to be amended, further work on the concept of history of safe use and guidance on the management of the risk assessment process."

The report does not recommend a wholesale change to EFSA's risk assessment process but states: *"As such, it is recognised in the foreseeable scenario for risk assessments that approaches to risk assessment will be based on using a comparative approach as a starting point, with differences to the current EFSA guidance to make the process effective at assuring the safety of plants with 'novel' GM traits."* There is no record of any of the recommended further work being undertaken, nor of any further steps being undertaken by EFSA to develop guidance for the risk assessment of nutrient-altered GM crops. No final guidance has been published or adopted.

It is unclear whether EFSA informed the European Parliament, the Commission and the Member States of the existence of Mandate Number M-2012-0084 or this expert report, as it is required to do under Article 32 of Regulation 178/2002. No correspondence on the subject is recorded in EFSA's Register of Questions. Thus, whilst it is clear that EFSA was aware in 2013 that its existing Guidance was not fit-for-purpose, it is unclear whether other EU bodies were informed of this before being requested to approve the authorisations granted in 2015. However, if the risk manager (DG SANCO, rather than EFSA) made the decision not to allow or require EFSA to proceed with developing specific Guidance before authorising nutrient-altered GM crops, this also undermines the scientific quality of the risk assessments (as outlined below) and the legal basis of the approvals.

The principle of transparency in EU food law (Article 9, Regulation 178/2002) also requires that there is open and transparent public consultation during the preparation, evaluation and revision of food law. No such consultation has taken place in relation to the development and detailing of a strategy for the assessment of nutritionally-altered GM crops.

Issues of conflicts-of-interest are raised by the fact that one of the research institutes commissioned to write the export report under Mandate Number M-2012-0084, Rothamsted Research, is involved in developing nutrient-altered GM crops, notably GM sativa with enhanced omega-3 oils (Rothamsted Research, 2015). The one altered-oil crop included as a future scenario in the report (Section 8) is a GM oil seed plant producing enhanced levels of long-chain polyunsaturated fatty acids (omega-3) oils such as DHA and EPA i.e. a product being developed through Rothamsted's own R&D. Several of the authors of this report therefore have a clear conflict-of-interest as they are employed by the institution hoping to commercialise this research, which could benefit financially from weak regulation of nutritionally-altered crops and minimal data requirements. Furthermore, the main overview articles cited in relation to developing a risk assessment process for nutritionally-altered GM crops are all written by industry authors (Constable et al., 2007; Glenn, 2007, 2008; ILSI, 2007), although these industry-affiliations are not noted in the text. The authors highlight a number of areas of significant scientific disagreement in their report and acknowledge that: *"It also became apparent from preliminary searches of the literature that the types of records sourced would not contain extensive amounts of numerical data, rather dialogue, and to some extent opinion from the author or risk assessment body regarding strategies for risk assessment"* (Section 2.1.1). In Section 7 (Foreseeable scenarios for risk assessment) they state: *"Please note that this section is based on the judgements and discussion of members of the project team…"*. The report states that it may not be considered as an output adopted by the Authority and it is clear that further steps should have been taken by EFSA to (i) complete the process it initiated to adopt new guidance; and (ii) ensure independence and transparency by, for example, commissioning further work from independent scientists, consulting with a wider range of stakeholders, conducting a public consultation, and keeping the relevant EU institutions fully informed.

In summary, EFSA has initiated but not completed a process of developing guidance for the assessment of GM crops with significantly altered nutritional content. As well as being incomplete, this process has not been independent or transparent. In the absence of this guidance, it is questionable whether approvals should have been granted for nutritionally-altered GM crops. The next section considers the safety implications of lack of risk assessment guidance for these crops.

1.2 Lack of a full nutritional/food safety assessment for oil-altered GM crops

EFSA has published scientific opinions on MON 87769 (EFSA Panel on Genetically Modified Organisms (GMO), 2014); MON 87705 (EFSA, 2013a, 2012); and soybean 305423 (EFSA, 2013b). Table 1 provides a summary of the genetic engineering of these products.

Table 1: Summary of genetic engineering of oil-altered GM crops approved for import as food and feed

Event name (Trade Name)	Company	Method	Traits	Gene introduced	Gene source	Product	Intended function
MON87769	Monsanto	Agrobacterium tumefaciens-mediated plant transformation	Modified oil/fatty acid	Pj.D6D	Primula juliae	delta 6 desaturase protein	desaturates certain endogenous fatty acids resulting in the production of stearidonic acid (SDA), an omega-3 fatty acid
				Nc.Fad3	Neurospora crassa	delta 15 desaturase protein	desaturates certain endogenous fatty acids resulting in the production of stearidonic acid (SDA), an omega-3 fatty acid
MON87705 (Vistive Gold)	Monsanto	Agrobacterium tumefaciens-mediated plant transformation	Glyphosate herbicide tolerance, Modified oil/fatty acid	fatb1-A (sense and antisense segments)	Glycine max	no functional enzyme is produced (production of FATB enzymes or acyl-acyl carrier protein thioesterases is suppressed by RNA interference)	decreases the transport of saturated fatty acids out of the plastid, thereby increasing their availability to desaturation to 18:1 oleic acid; reduces the levels of saturated fatty acids and increases the levels of 18:1 oleic acid
				fad2-1A (sense and antisense)	Glycine max	no functional enzyme is produced (production of delta-12 desaturase enzyme is suppressed by RNA interference)	reduces desaturation of 18:1 oleic acid to 18:2 linoleic acid; increases the levels of monounsaturated oleic acid and decreases the levels of saturated linoleic acid in the seed
				cp4 epsps (aroA:CP4)	Agrobacterium tumefaciens strain CP4	herbicide tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
Soybean 305423 (Plenish)	DuPont (Pioneer Hi-Bred International Inc.)	Microparticle bombardment of plant cells or tissue	Sulfonylurea herbicide tolerance, Modified oil/fatty acid	gm-hra	Glycine max	modified acetolactate synthase (ALS) enzyme	confers tolerance to applications of sulfonylurea – based herbicides
				gm-fad2-1 (partial sequence)	Glycine max	no functional enzyme is produced (expression of the endogenous fad2-1 gene encoding omega-6 desaturase enzyme was suppressed by the partial gm-fad2-1 gene fragment)	blocks the formation of linoleic acid from oleic acid (by silencing the fad2-1 gene) and allows accumulation of oleic acid in the seed

These three products which have been authorised for import as food or feed feature significant and complex changes in nutrient content, some of which are unintentional:

- 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 (ALA); 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). The compositional analysis also revealed increased protein and differences in the levels of amino acids. For the processed oil, 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 also 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). Small quantities of trans-ALA were present in all types of refined, bleached and deodorised soybean oil. LA in protein isolate from soybean MON 87769 was reduced, and trans-ALA and ALA increased. The fat phase of the protein isolate produced from soybean MON 87769 also contained SDA, GLA and trans-SDA. The crude lecithin derived from soybean MON 87769 contained SDA, GLA and trans-SDA, which are usually not detected in lecithin from conventional soybeans, and the level of linoleic acid (C18:2) was significantly reduced.
- MON 87705 differs from the conventional counterpart in the fatty acid profile (proportion of (C18:1) oleic acid increased and proportions of (C18:2) linoleic acid and (C16:0) palmitic acid decreased) in seeds and the presence of the CP4 EPSPS protein. Smaller although significant decreases in stearic acid, linolenic acid, arachidic acid and behenic acid and an increase in eicosenoic acid were also observed. The intended effects of the genetic modification and the effects on the fatty acid pattern seen in the analysis of unprocessed soybean seeds were also reflected in the composition of derived oil and additional differences were seen in heptadecenoic acid (C17:1 9c) and octadecadienoic acid (C18:2 6c, 9c).
- In soybean 305423, the conversion of oleic acid to linoleic acid is inhibited and the oleic acid level is elevated. Since linolenic acid is produced from linoleic acid, linolenic acid content is also decreased in soybean 305423. Some of the observed differences of the fatty acid profile are consistent with the intended effect of the genetic modification, i.e. an increase in oleic acid at the expense of PUFA, but changes in the levels of odd chain fatty acids are an unintended effect probably caused by the introduction of the ALS enzyme. Other parameters (calcium, zinc and glycitin and related total glycitein equivalents) also showed non-equivalence. Data was not provided for the compositional content of derived oil, despite this being the main product destined for human consumption.

In all three cases the nutritional content of the soybeans is clearly not (and is acknowledged not to be) “substantially equivalent” to conventional soybeans. In addition, the nutritional changes are complex and not limited to a single nutrient.

The application of herbicides may further alter chemical composition. This means it is also important to assess the nutrient content of the soybeans when the herbicide is applied (see the subreport on herbicide-tolerant GM crops).

1.2.1 The overall effect on health of these nutritional changes is poorly understood

Article 6 of Regulation (EC) No. 1829/2003 requires applications to include a systematic review of studies published in the scientific literature and studies performed by the applicant on the potential effects on human and animal health of the GM food.

For MON 87769, the applicant cites published studies in humans and animals of the four fatty acids found in higher amounts in MON 87769 than in conventional soybean: SDA, GLA, 9c,12c,15t trans-ALA (18:3) and 6c,9c,12c,15t trans-SDA (Section 5.1.2.3 of EFSA's Scientific Opinion). 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. However, 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. Further, these studies are not directly relevant to the product being assessed.

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 EFSA's Scientific Opinion on this product states that the rationale for developing MON 87769 is that this may result in a more efficient synthesis of the higher chain-length PUFAs (EPA and DPA). There is some evidence of this from a study conducted by Monsanto and Southern Illinois University in rats (Casey et al., 2013) and a subsequent clinical trial of SDA soybean oil from biotechnology-derived soybean MON 87769 in 252 overweight human subjects (Lemke et al., 2010), although the latter is not a large-scale trial.

However, studies on the reduced linoleic acid (LA) levels in soybean MON 87769 are not included in the literature review and nor are studies on the intended impact of the product on omega-3 levels (which the applicant wished to refer to on the label, see Section 1.2.7 below). These omissions from the literature review for MON 87769 are important because the scientific literature includes evidence of potential harm to health from omega-3 fatty acids (increased prostate cancer risk) (Brasky et al., 2011, 2013; Chua et al., 2013). Although EFSA claims to have subsequently considered these papers elsewhere (EU Commission 2018), they were not cited in the assessment of the GMO application, whereas in the context of an application for the use of algal oil as an ingredient in novel foods, a three page discussion was deemed necessary (EFSA, 2014b). This inconsistency is inexcusable, as genetically engineering these changes into the crop itself, as opposed to supplying a particular ingredient, should require an assessment to the highest standards.

Despite many claims to the contrary, there is no conclusive evidence of health benefit from increased omega-3 (Fezeu et al., 2014; Hooper et al., 2006; Kwak et al., 2012; Rizos et al., 2012).

No literature review of health impacts is included for the altered fatty acid content of the soybeans in the other applications. The literature includes evidence of potential harm to health from low linolenic acid (as reported in soybean 305423 and MON 87705) (Djoussé et al., 2003). In relation to MON 87705 and soybean 305423, which increase oleic acid (a mono-saturated fat or MUFA), a literature review would also have revealed:

- Studies suggesting a link between oleic acid/MUFAs and breast cancer (Chajès et al., 2008;

Saadatian-Elahi et al., 2004); and
- Studies suggesting a link between MUFAs and poor memory function (Gibson et al., 2013).

Again, there is no consensus in the literature on the claimed benefits of MUFAs for cardiovascular disease risk (Schwingshackl and Hoffmann, 2012).

Although these studies do not provide definitive proof that these altered nutrients are harmful, they highlight the need for a precautionary approach. These findings are important because they suggest that the regulatory requirement to protect human health requires studies which are adequate to identify endpoints such as cancer risk or memory loss in humans. Identifying such endpoints normally requires long-term clinical studies in humans.

A literature review would also have identified 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, young children, pregnant women and people with specific health conditions (discussed further in Section 1.2.4 below). This is a particularly serious omission because of the importance of the role of fatty acids in brain development and functioning.

Had specific Guidance for nutritionally-altered GM crops been in place, it is likely that all applicants would have been aware of the requirement for a comprehensive literature review of the health impacts of the altered nutrients in their products and would have identified a number of likely or potential harms to human health. This would have allowed hypotheses regarding risks to human health to be developed and tested in the remainder of the assessment.

1.2.2 Gene-environment interactions and herbicide application will affect nutrient content

Environment and gene-environment interactions (GxE) are known to have important effects on nutrient (including fatty acid) composition of soybeans (Whent et al., 2009), leading to significant alterations in fatty acid content in different environmental conditions (e.g. temperature and rainfall) and such effects can vary at different developmental stages (Han et al., 2011). It is therefore essential that data on nutrient composition of the edible parts of the plant is obtained from a wide variety of agronomic conditions, representative of expected growing conditions (including herbicide treatment).

The data provided in the three relevant EFSA Opinions is inconsistent between applications and in some cases clearly inadequate to deal with the case of nutrient-altered crops. Specifically, according to the relevant EFSA opinions:
- 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 over 2 years 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.
- The comparative analyses for MON87705 were carried out at 5 different geographical sites in 2007/08 (including data from the US and Chile) and at 5 sites in the USA in 2008 (one USA site was excluded from the analysis).
- For soybean 30542, analysis was undertaken of the GM-HRA protein and fatty acid profile of seeds collected across several locations in Chile, Argentina and the USA. Field trials for compositional data were performed at six locations in the USA and Canada in 2005 and at

six locations during the season 2005–2006 in Chile and Argentina. An additional comparative field trial was performed at ten sites within soybean cultivation areas in the USA in 2011.

Of the three products, MON87769 is already authorised for commercial cultivation in Canada and the USA (ISAAA, undated). At minimum, data from Canadian trial sites is also required to establish the nutritional composition for this soybean due to likely very different cultivation conditions (e.g. climate, soil types).

Nutrient-altered GM crops are a special category of GM crops in which the dose of the altered nutrients and perhaps other biochemical may be highly dependent on environmental conditions. Further, health effects in humans and other species may relate closely to the dose. Therefore it is inappropriate for EFSA to rely on its standard guidance for GM crops: specific guidance should have been developed to enable the composition of nutrient-altered GM crops to be determined in a wide variety of conditions.

Monsanto's MON 87705 soybean is a low-linoleic, high-oleic soybean which is also tolerant to the herbicide glyphosate. However, glyphosate application can influence seed composition by altering nitrogen metabolism, leading to lower linolenic acid and higher oleic acid in treated soybeans (Bellaloui et al., 2008, 2009). This means it is also important to assess the nutrient content of the soybeans when the herbicide is applied.

The lack of Guidance for nutrient-altered crops means that EFSA has failed to specify requirements for composition data for nutrient-altered crops which take account of the importance of gene-environment interactions and herbicide applications.

1.2.3 Lack of full nutritional/food safety analysis

Regulation (EC) No 1829/2003 defines a conventional counterpart as *"a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use"* (Article 2.12). The underlying assumption of the comparative approach is that traditionally cultivated crops have a history of safe use for consumers and/or domesticated animals. The range of natural variation is estimated from a set of non-GM reference varieties and this allows comparisons of the GM plant with a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use.

However, all three plants that are the subject of this complaint have nutritionally-altered components that fall outside this natural variation (as detailed above) and therefore all three products lack a history of safe use. EFSA's Guidance for risk assessment of food and feed from genetically modified plants (EFSA Panel on Genetically Modified Organisms (GMO), 2011b) states (Section 3.1.3):
"Where no comparator can be identified, a comparative risk assessment cannot be made and a comprehensive safety and nutritional assessment of the GM plant and derived food and feed itself should be carried out. This would, for instance, be the case where the food and/or feed derived from a GM plant is not closely related to a food and/or a feed with a history of safe use, or where a specific trait or specific traits are introduced with the intention of changing significantly the composition of the plant".
And:
"In case an appropriate comparator is not available, a comparative assessment cannot be made and, ***therefore a safety and nutritional assessment of the GM plant and derived products should***

be carried out as for other novel foods. *In such cases, the elements to be considered for the risk assessment are the same as those listed in Section 2.3."* [Emphasis added]

In relation to nutritionally altered crops, Codex guidance states (Codex Alimentarius, 2003):
"48. The assessment of possible compositional changes to key nutrients, which should be conducted for all recombinant-DNA plants, has already been addressed under 'Compositional analyses of key components'. ***However, foods derived from recombinant-DNA plants that have undergone modification to intentionally alter nutritional quality or functionality should be subjected to additional nutritional assessment to assess the consequences of the changes and whether the nutrient intakes are likely to be altered by the introduction of such foods into the food supply.*** *A detailed presentation of issues to be considered can be found in Annex 2 to this document."* [Emphasis added].

For the products authorised to date, the focus of EFSA's assessment is on comparing the main altered nutrient with standard dietary recommendations. However, this falls short of a comprehensive nutritional assessment. Information from each of the relevant Scientific Opinions is summarised below.

For MON 87769:

- The applicant used information from the United Kingdom (UK) National Diet and Nutrition Survey (adults 19–64 years old) and the US FDA information on serving sizes to calculated the intake of SDA-rich soybean oil and SDA. Making various assumptions, the applicant then calculated that the estimated mean per capita intake of SDA from the suggested use of SDA soybean oil would be equivalent to a dietary intake of around 0.4–0.8 g EPA/person/day and would result in a cumulative estimated intake below the level of 5 g/day of supplemental combinations of EPA and DHA and of 1.8 g of EPA alone per day, which were considered to be safe for adults by EFSA.
- The applicant also used the UK National Diet and Nutrition Survey to estimate the impact of replacing presently used vegetable oils in foods with SDA-rich soybean oil on the intake of other fatty acids, concluding that the dietary intake of n-3 PUFAs would increase by 2.70–2.85 g/day, whereas the intake of n-6 PUFAs would decrease by 0.85–0.62 g/day and the total saturated fatty acid intake would increase by 0.54–0.79 g/day. They conclude that the estimated reduction in LA intake is without concern with regard to the adequate intake (AI) for linoleic acid (LA) established by EFSA.
- Upon request, the applicant performed an additional assessment of the changes in fatty acid intake of consumers owing to substitution of conventional soybeans in soybean foods including soybean oil, with soybeans MON 87769, using consumption data from the UK, France and Denmark. The greatest changes occurred in the UK and consisted of an increase in the ALA intake of 0.5 g/day, in the SDA intake of 3.4 g/day, in the GLA intake of 1.1 g/day and in the palmitic acid intake of 0.17 g/day, whilst the intake of LA decreased by 4.9 g/day and that of oleic acid by 0.5 g/day. This LA intake would correspond to about 3 E%, which is below the AI set by EFSA.
- A twenty-eight-day repeated dose toxicity study, a sub-chronic toxicity study, and a one generation reproductive toxicity study were also conducted with soybean oil in Sprague–Dawley rats (Hammond et al., 2008).

For MON 87705:

- The nutritional assessment is focused on the intended increase of oleic acid (C18:1) and the accompanying decreases of linoleic acid (C18:2) and palmitic acid (C16:0), of which the levels were outside the ranges of the natural variation. The mean per capita intake of soybean oil from the target foods is estimated for adult males and females only. EFSA's Opinion states that there would be a substantial increase in oleic acid intake, while the PUFA intake would be markedly reduced: however levels would remain within the range of dietary recommendations for both n-3 PUFA and n-6 PUFA.
- In response to a further request, the applicant provided exposure assessments based on total and partial substitutions of conventional soybean, rapeseed and sunflower oils with the soybean MON87705 oil in foods (salad dressings, margarines and spreads, mayonnaise, crackers and salty snacks and soybean/rapeseed/sunflower oils in processed foods). The average and upper percentile intakes (expressed as g/day and as E % of the total diet) of five fatty acids (palmitic, stearic, oleic, linoleic and α-linolenic acid) arising from a total substitution of other oils by soybean MON 87705 oil were estimated, and the likely changes in total fatty acid consumption from the whole diet were calculated. EFSA has not set a dietary reference value (DRV) for SFAs or MUFAs. EFSA has proposed an adequate intake (AI) for ALA of 0.5 E%: the n-3 PUFA intake, around 1 E % at baseline in men and women, would fall by about 5% in the substitution scenario. EFSA has proposed an AI for linoleic acid of 4 E %: intakes of n-6 PUFA for adults would fall by around 40% from above to below this level. Only adults are considered in the assessment.
- Diets containing defatted meal from soybean MON 87705 were tested in rats: no animal studies were undertaken for derived oil, which is the main product destined for human consumption.

For soybean 305423:

- Dietary intakes were estimated for five fatty acid groups (saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), n-6 PUFA, n-3 PUFA and trans fatty acids (TFA)). Average and upper percentile intake amounts of the relevant food groups containing soybean oil were calculated and compared with reference values and normal dietary intakes. EFSA has not set a dietary reference value for SFA or MUFA. EFSA has proposed an adequate intake (AI) for linoleic acid (LA, the main dietary n-6 PUFA in the human diet) of 4 E %: reduction below the AI was observed for toddlers, children and teenagers (3.2–3.8 E %). EFSA has proposed an AI for Alpha-linolenic acid (ALA, the main dietary n-3 PUFA) of 0.5 E %: this was exceeded in all subgroups studied and replacement of vegetable oils with soybean 305423 oil was calculated to result in an increase of the n-3 PUFA consumption for all age groups.
- On request of the EFSA GMO Panel, the applicant provided an exposure assessment for the odd chain fatty acids: however no reference values have been set for these unintended changes. The Scientific Opinion concludes that full replacement of vegetable oils with oil derived from soybean 305423 would not change substantially the average intake of SFA and n-3 PUFA, but would increase MUFA and odd chain fatty acids, and decrease n-6 PUFA intake. It states that these changes are small and without impact on health and nutrition.
- For animal studies, dehulled, fat-extracted toasted soybean meal was the principal product tested, with small amounts of hulls and/or oil added in rat and chicken studies i.e. these studies focused on the product likely to be consumed by animals,

not on the oil for human consumption.

Further, the effects of herbicide-treatment were not included in these studies.

The EFSA GMO Panel notes that vegetable oil consumption varies considerably between European Countries and may be much higher outside the UK, however UK data are relied on in all three assessments and estimates of impacts on other European populations are not considered, except for MON 87769 which uses some data from France and Denmark.

Codex Guidance states (para 50):
"The use of plant breeding, including in vitro nucleic acid techniques, to change nutrient levels in crops can result in broad changes to the nutrient profile in two ways. The intended modification in plant constituents could change the overall nutrient profile of the plant product and this change could affect the nutritional status of individuals consuming the food. Unexpected alterations in nutrients could have the same effect. ***Although the recombinant-DNA plant components may be individually assessed as safe, the impact of the change on the overall nutrient profile should be determined.****"* [Emphasis added]. However, all the assessments take a nutrient-by-nutrient approach and fail to assess the impact on health of the overall nutrient profile.

Codex guidance states (para 49): *"It is also important to ascertain to what extent the modified nutrient is bioavailable and remains stable with time, processing and storage"*. However, bioavailability studies have not been included for any of the three soybeans.

Codex guidance also states (para 53):
"Some foods may require additional testing. For example, animal feeding studies may be warranted for foods derived from recombinant-DNA plants if changes in the bioavailability of nutrients are expected or if the composition is not comparable to conventional foods. Also, foods designed for health benefits may require specific nutritional, toxicological or other appropriate studies. If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal studies could be requested on the whole foods." However, only MON 87769 soybean oil has been tested in rats: the other animal studies in the applications focus on testing animal feed which has minimal oil content and is not comparable to the main product destined for the human diet. Further, the rat studies for MON 87769 soybean oil are inadequate to test long-term impacts in humans. 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 and appropriate endpoints such as cancer risk in humans (which should have been identified by the literature review, see Section 1.2.1) were not considered.

Subsequently, the first study of the metabolic effects of genetically modified high oleic soybean oil in mice was presented at a conference. The authors designed a parallel diet in which the regular soybean oil was replaced, on a per gram basis, with GM high oleic acid soybean oil (Plenish soybean 305423) (Deol et al., 2015). To the authors' surprise this diet induced weight gain and fatty liver essentially identical to that of the unmodified soybean, although the mice remained insulin sensitive and had less adipose tissue. The results indicate that LA may contribute to insulin resistance and adiposity but that another as yet unidentified component of the soybean oil affects the liver and overall weight gain. The authors conclude that a thorough understanding of the metabolic effects of the GM soybean oil is essential before the adoption of yet another dietary trend that could have long lasting and impactful health consequences. However, EFSA has not required applicants to submit this study or any comparable studies for any of the soybeans. A published journal paper by the same group has since reported that whilst soybean 305423 induced less insulin

resistance than conventional soybean oil, it also resulted in liver enlargement and dysfunction and may therefore induce detrimental health effects in terms of liver function (Deol et al., 2017).

Monsanto has conducted a clinical trial of SDA soybean oil from biotechnology-derived soybean MON 87769 in humans (Lemke et al., 2010, cited in Section 1.2.1 above and in EFSA's Scientific Opinion) but (despite the title of the paper) this does not address the safety of the nutritional changes. For the other two products, data from human trials does not appear to be available.

For MON87769, the applicant focuses on the intended physiological consequence of consuming the soybeans i.e. enhanced synthesis of the long chain omega-3 polyunsaturated fatty acids (PUFAs) EPA) and DHA. The EFSA Opinion relies heavily on the fact that EFSA 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 EFSA report which established the AI is out of date (EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA), 2010) and more recent studies must be included (e.g. studies suggesting increased prostate cancer risk as cited above in Section 1.2.1); (ii) it does not consider population subgroups who may be particularly affected by changes in the fatty acid profile of their food (discussed further below in Section 1.2.4); (iii) it requires an extrapolation, based on limited data, of the impacts of the product on EPA+DHA and ignores other nutritional changes (contrary to Codex Guidance cited above) (iii) it is not applicable to GMO foods which require a full safety assessment under Regulation (EC) No. 1829/2003.

In summary, the lack of Guidance for the assessment of nutritionally-altered GM crops has resulted in an inadequate assessment process which fails to protect human health. There are also major inconsistencies between the information supplied for different products.

1.2.4 Potentially vulnerable subgroups need to be considered

Article 14(4) of Regulation 178/2002 states:
4. In determining whether any food is injurious to health, regard shall be had:
(a) not only to the probable immediate and/or short-term and/or long-term effects of that food on the health of a person consuming it, but also on subsequent generations;
(b) to the probable cumulative toxic effects;
*(c**) **to the particular health sensitivities of a specific category of consumers where the food is intended for that category of consumers***." [Emphasis added]

Implementing Regulation 503/2013 notes (Annex II, Part II, 1.6.3):
"*Genetically modified foods modified to provide additional health benefits to the consumer as compared to conventional foods, **may benefit specific populations or subpopulations while others may be at risk from the same food**. In cases where an altered bioavailability needs to be established and may raise concern for subpopulation(s), the level of the nutrient in the food shall be determined, taking into account all the different forms of the compound. The methods to test for bioavailability shall be selected on a case-by-case basis depending on the nutrient or other constituent, the food containing these constituents, as well as the health, nutritional status and dietary practices of the specific population(s) anticipated to consume the food*". [Emphasis added]

Codex Guidance also notes (Annex 2): "*Foods derived from recombinant-DNA plants modified for nutritional or health benefits may benefit certain populations/sub populations, while other populations/sub populations may be at risk from the same food*" and states (point 49):
"*Attention should be paid to the particular physiological characteristics and metabolic requirements of specific population groups such as infants, children, pregnant and lactating women,*

the elderly and those with chronic diseases or compromised immune systems. Based on the analysis of nutritional impacts and the dietary needs of specific population subgroups, additional nutritional assessments may be necessary".

The expert report commissioned by EFSA states (Section 3.8):
"*Exposure assessment should also consider population differences that may result in segregated risks. This applies also to vulnerable subsets/ at-risk groups of a population, including diabetics, nursing mothers, pregnant women, children, and the elderly, which should be separately evaluated for exposure, to determine whether the GM food crop may pose a separate risk to them*".

Thus, EFSA Guidance and Codex Guidelines require population subgroups to be considered in the nutritional and safety assessment. As well as categories by age, this should include other subgroups whose nutrient requirements may be different from the general population.

However, data provided for the Scientific Opinions is inconsistent between applications and too limited to assess risks to vulnerable subpopulations:

- In the dietary assessment for MON 87769, only average adult intakes are considered.
- In the main and supplementary assessment for soybean MON 87705 oil is assumed to replace conventional soybean, rapeseed and sunflower oils in foods consumed by the adult UK population only. No impacts on subpopulations are considered.
- For soybean 305423, consumption data are taken from the UK National Diet and Nutrition Survey (NDNS) of 2008–2010 and the sub-populations considered are toddlers (1–3 years), children (4–10 years), teenagers (11–18 years), adults (19–64 years) and the elderly (≥ 65 years).

No studies for any of the soybeans have been included for pregnant or lactating women. There is extensive documentation that foetuses and new-born babies are the most sensitive to diet. Changing the lipid composition of the developing brain by eating food with altered fatty acid compositions may have adverse effects, particularly in some genetic conditions (see below). In addition, it is very likely that nutritionally modified plants contain molecules related to the intended products that have unknown effects (Schubert, 2008).

Bioavailability studies in vulnerable subpopulations have not been included for any of the three soybeans.

EFSA acknowledges that, in the three cases, dietary intake estimates were made for adults (19-64 years old) and, in only one case (305423 soybean), also for other age groups (< 19 years old) and in no case for pregnant and lactating women and the elderly (EU Commission 2018). However, it states that no such assessment is necessary (EU Commission 2018).

Regarding concerns about the decrease in linoleic acid, EFSA states that reduction below the adequate intake of 4 E% was observed for toddlers, children and teenagers (3.2-3.8 E %), but then states that the EFSA GMO Panel is of the opinion that this is not a matter of concern, as linoleic acid deficiency symptoms have not been observed at intakes > 1 E % (EU Commission 2018). Thus, rather than paying particular attention to the needs of vulnerable subgroups, the method of comparison with adequate intakes has been discarded when it might have led to refusal of the application.

Persons with chronic diseases have also been neglected. For example, there are a number of monogenic genetic disorders, e.g. 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, 2005). The implications of the low linoleic acid levels observed in soybean MON 87769 and soybean 305423 should have been considered for these vulnerable groups. Propionic acidemia and methylmalonic aciduria are genetic disorders of propionate catabolism which result in abnormality of odd-numbered Long-Chain Fatty Acids (Wendel, 1989). No studies are available to assess the health impacts on this group of the unexpected increases in odd-numbered Long-Chain Fatty Acids in soybean 305423.

However, the lack of such studies also impacts on the failure to meet labelling requirements (see below) since sufficient information is not available to consumers and the medical profession to make informed decisions about the possible inclusion of these products in their diets.

1.2.5 Impacts of food processing need to be considered for all food types

Codex guidance states (para 47):
"*The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants should also be considered. For example, alterations could occur in the heat stability of an endogenous toxicant or the bioavailability of an important nutrient after processing. Information should therefore be provided describing the processing conditions used in the production of a food ingredient from the plant. For example, in the case of vegetable oil, information should be provided on the extraction process and any subsequent refining steps*".

However, not all forms of the processed soybeans were fully tested before approval of the products which are the subject of this complaint:

- For MON 87769, soybeans were harvested from two of the five sites in the USA in 2006 in order to perform compositional analyses on processed fractions, including defatted and toasted meal; refined, bleached and deodorised oil; protein isolate; and crude lecithin. The soybean meal was analysed for proximates, fibre fractions, amino acids, fatty acids, phytic acid and trypsin inhibitors, the soybean oil for fatty acids and vitamin E, the protein isolate for amino acids, fatty acids and moisture and, finally, crude lecithin for fatty acids and phosphatides.
- For soybean MON 87705, the seeds were processed into refined bleached deodorised (RBD) oil, isolated soy protein, toasted defatted meal and crude lecithin for further composition tests. RBD oils were analysed for fatty acid composition and vitamin E, isolated soy protein was analysed for amino acids and crude lecithin was analysed for phosphatides. Seed samples to prepare soybean processed fractions were collected from field trials where MON 87705 and the conventional counterpart A3525 were grown in replicated plots at two sites in the USA during the 2007 growing season. The intended effects of the genetic modification and the effects on the fatty acid pattern seen in the analysis of unprocessed soybean seeds were also reflected in the composition of RBD oil. The main product for human use is soybean oil. However, EFSA's Scientific Opinion notes that in addition, soybean is used for the production of soybean milk, protein concentrates, flour, sprouts, baked or roasted soybeans, tofu, soybean sauce and other products for human consumption. No analyses were conducted for these products.
- For soybean 305423, the main product for human consumption is the oil, and other products for human consumption were not considered in EFSA's Scientific Opinion. For the exposure assessment, food items considered are the targeted foods (fried fish, meat, potatoes, vegetables and other fried foods, home-use; and from spray applications savoury snacks and crackers) and other foods (salad dressings, margarines and spread, mayonnaise). The fatty acid composition of the oil from soybean 305423 is taken from that of the unprocessed seeds

from the field trial of 2011 and the oil is assumed to fully replace vegetable oils in the individual food items.

In all cases, the nutritional and safety assessment for humans is focused on use of soybean oil. However, there is inconsistency because the fatty acid composition of the oil itself (only the seed) is not used for soybean 305423 and the effects of processing are therefore not considered in this case. In addition, EFSA's Opinion for MON 87769 (Section 5.1.2.3 (c)) states that it is assumed that trans-SDA is mainly formed by trans-isomerisation of unsaturated fatty acids during the processing of the oil: but no specific studies have looked at the effects of consuming trans-SDA.

Other products for human consumption including soybean milk, protein concentrates, flour, sprouts, baked or roasted soybeans, tofu and soybean sauce are not assessed for their fatty acid content or health impacts for any of the three soybeans.

On request from the EFSA GMO Panel, the applicant supplied information on the oxidative stability of the SDA enriched oil obtained from soybean MON 87769. However, this information does not appear to have been required from other applicants.

Studies on the stability of oil-altered foodstuffs are important because lipid/ fatty acid oxidation is a major cause of foods with high oil content 'going bad'. However, no such studies have been provided.

In the absence of Guidance for the assessment of nutrient-altered crops, none of the applicants have tested all the forms of the soybean products which may be consumed by humans. This is inconsistent with the risk assessment of novel foods, as described in the 2012 expert report commissioned by EFSA, for which the starting point is considering the processing the crop would undergo and the products which would be manufactured or marketed to consumers.

1.2.6 Use of the GM crop as feed can alter nutrient content of (unlabelled) meat and dairy products

EU food law states (Article 15, Regulation 178/2002) that feed shall be deemed to be unsafe for its intended use if it is considered to:
— have an adverse effect on human or animal health;
— make the food derived from food-producing animals unsafe for human consumption.

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 (Section 5.1.5.2 of the relevant Scientific Opinion). However, the Panel did not consider the nutritional impact from consuming products of animal origin derived from animals fed whole fat MON 87769 or its oil on consumers. In fact, none of the three scientific opinions provided by EFSA on nutritionally-altered soybeans assess the impact on the nutritional content of meat, milk or eggs. Therefore the opinions do not include the necessary assessment of whether food derived from food-producing animals fed on any of the three GM soybeans is safe for human consumption.

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 et al., 2010) as has already been attempted using supplements (Glasser et al., 2008). Since potential food and feed uses have not been restricted, this use should fall within the scope of the assessments. Further, it is likely that a similar approach could be applied to meat and eggs where diet is known to affect fat composition (Berthelot et al., 2010;

Oliveira et al., 2011). Since such uses can be anticipated, nutrient (and anti-nutrient) composition should have been required for meat, milk and eggs from animals fed on all three nutrient-altered soybeans.

In its comments on MON 87769, Germany notes that the applicant should specify whether whole soybean MON 87769, processed material or the derived SDA-rich oil are intended to be used as animal feed and whether impacts on the food (e. g. meat or milk) derived from animals which were fed these materials are expected. However, EFSA responded that foods and feeds derived from animals fed soybean MON 87769, feed containing or consisting of soybean MON 87769 and feed produced from this soybean, are not within the application. It is hard to see how this response is consistent with the requirements of Article 15, Regulation 178/2002.

Subsequently, a study has been published which reports that Plenish soybean (soybean 305423) diets increased milk fat concentration and tended to increase fat yield, decrease feed efficiency, and modify milk fatty acid profile in lactating dairy cows (Lopes et al., 2017). The study involved 15 Holstein cows and the soybean sources were conventional, high-linoleic-acid variety extruded soybean meal; extruded Plenish, high-oleic-acid variety soybean meal; and whole, heated Plenish soybeans. The diets were designed to maximize the potential milk composition effects of the soybean sources while minimizing negative effects on rumen fermentation and Dry Matter Intake (DMI). The Plenish diets increased milk fat concentration and tended to increase milk fat yield. Generally, the Plenish diets increased mono-unsaturated (mostly *cis*-9 18:1) and decreased polyunsaturated, total *trans*-, and conjugated linoleic fatty acids concentrations in milk fat. The Plenish diets also altered the bacteria in the rumen, decreasing the prevalence of *Ruminococcus* and increasing that of *Eubacterium* and *Treponema* in whole ruminal contents.

This and similar studies should have been included as part of EFSA's risk assessment process, in order to make an informed assessment of the nutritional impact of these GM crops on meat and dairy products produced using these GM soybeans in feed. . However, EFSA argues that, although the deliberate introduction of modified soybean oil into animal diet with intention of changing the fatty acid profile of animal products is possible, to date there has been no commercial uptake and consequently, at present no human exposure assessment of this nature can be made (EU Commission 2018).

This important issue is unlikely to have been missed had EFSA developed Guidance for the assessment of nutrient-altered GM crops.

1.2.7 Food labelling proposals are inadequate to provide sufficient information for consumers

The lack of Guidance for the risk assessment of nutrient-altered foods has also resulted in a failure to appreciate the need to provide adequate information regarding the new nutritional content on labels.

EU food law aims at the prevention of practices which may mislead the consumer (Article 8, Regulation 178/2002) and Regulation 1830/2003 requires products consisting of or containing GMOs to be labelled and to be traceable via a unique identifier provided to the operator receiving or placing a product on the market (but not to the consumer). This Regulation does not specify detailed labelling requirements for nutritionally-altered crops that may pose risk to specific subcategories of consumer. However, Article 14(3) of Regulation 178/2002 states:
"*3. In determining whether any food is unsafe, regard shall be had:*

(a) to the normal conditions of use of the food by the consumer and at each stage of production, processing and distribution, and
(b) ***to the information provided to the consumer, including information on the label, or other information generally available to the consumer concerning the avoidance of specific adverse health effects from a particular food or category of foods***." [emphasis added].

Regulation (EC) 1829/2003 Recital (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*". [emphasis added].

Article 5 (1f) of Regulation 1829/2003 requires either an analysis, supported by appropriate information and data, showing that the characteristics of the food are not different from those of its conventional counterpart, having regard to the accepted limits of natural variations for such characteristics and to the criteria specified in Article 13(2)(a), or a proposal for labelling the food in accordance with Article 13(2)(a) and (3). Since the applicants accept the three soybeans are different from their conventional counterparts, labels have been proposed for all three products.

Article 13(2 and 3) of Regulation 1829/2003 state:
"*2. In addition to the labelling requirements referred to in paragraph 1, the labelling shall also mention any characteristic or property, as specified in the authorisation, in the following cases:*
(a) where a food is different from its conventional counterpart as regards the following characteristics or properties:
(i) composition;
(ii) nutritional value or nutritional effects;
(iii) intended use of the food;
(iv) implications for the health of certain sections of the population;
(b) where a food may give rise to ethical or religious concerns.
3. In addition to the labelling requirements referred to in paragraph 1 and as specified in the authorisation, the labelling of foods falling within the scope of this Section which do not have a conventional counterpart shall contain appropriate information about the nature and the characteristics of the foods concerned."
Article 14 highlights that detailed rules for implementing this Section, amongst other things regarding the measures necessary for operators to comply with the labelling requirements, may be adopted in accordance with the procedure referred to in Article 35(2). However, no such detailed rules have been adopted for nutrient-altered GM crops.

As a result of the lack of detailed rules, the proposed labelling does not conform to the legal requirements for any of the three soybeans which are the subject of this complaint, because (i) information is not provided about all the characteristics and properties that render the food or feed different from its natural counterpart; and (ii) no account as has been taken of the differing nutritional needs of different sections of the population, particularly children and those with metabolic disorders who may be adversely affected by altered nutrient content. More specifically:

- For MON87769, 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. Further, EFSA states that the proposal of the applicant to include the mention of "omega-3" on the label of

soybean MON 87769 was not accepted, as it constitutes a nutrition claim (EU Commission 2018). Commission Implementing Decision (EU) 2015/686 of 24 April 2015 states that the words ‘with stearidonic acid’ shall appear after the name of the organism on the label or, where appropriate, in the documents accompanying the products. However, to meet legal requirements 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).

- For MON 87705, the labelling proposal “increased oleic acid oil produced from genetically modified soybean” is inadequate because it fails to detail all the changes in the fatty acid profile, including the reduction in linoleic acid (LA). Commission Implementing Decision (EU) 2015/696 of 24 April 2015 states the words ‘with increased monounsaturated fat and reduced polyunsaturated fat’ shall appear after the name of the organism on the label or, where appropriate, in the documents accompanying the products. However, numerous GM soybeans with altered fatty acid profiles are in the GM industry pipeline with a wide variety of properties (Wilson, 2012). These products all have different fatty acid profiles and molecular characterisations and several could be described as having increased monounsaturated fat and reduced polyunsaturated fat, despite having substantially different fatty acid profiles (and in some cases other altered nutrients).
- For soybean 305423 the labelling proposal “genetically modified soybean with altered fatty acid profile” is inadequate for the same reason, as it also provides inadequate information on the nutritional changes including the reduction in linoleic acid (LA). Commission Implementing Decision (EU) 2015/698 of 24 April 2015 states that the words ‘with increased monounsaturated fat and reduced polyunsaturated fat’ shall appear after the name of the organism on the label or, where appropriate, in the documents accompanying the products. However, this implies equivalence with MON 97705, despite different fatty acid profiles.

For all three soybean products, it is particularly important that consumers are warned about low linoleic acid, given the potentially adverse effects of this nutritional change and the existence of vulnerable subgroups with Fatty Acid Metabolism Disorders (as described above). Consumer information is also important because a reduction below adequate intake (AI) for linoleic acid was observed for toddlers, children and teenagers in the assessment for soybean 305423 (children were wrongly omitted from the assessments for the other soybeans).

The failure to undertake comprehensive nutritional and safety assessments for vulnerable subgroups (as described above) also means there is inadequate information on which to base these labelling proposals as in most cases relevant subgroups (such as pregnant mothers) have not been considered.

To meet the legal requirements, 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 necessary 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, or high oleic acid and breast 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. Subgroups of consumers (e.g. children or those suffering from a particular metabolic disorder) may find health problems are caused by some fatty acid profiles but not others (as described above). They may therefore wish (or need) to avoid specific fatty acids or groups of fatty acids.

Detailed consumer information regarding specific products is essential to allow 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.

1. For MON87769, the proposed label "contains genetically modified soybean containing SDA omega-3 oil" also conflicts with food claims legislation, although this label application was subsequently refused for this reason. Whilst the risks of GM crops are considered by EFSA under Regulation 1829/2003, claims about the health benefits of products may be added to labels on a voluntary basis under Regulation 1924/2006. Under Regulation (EC) No. 1924/2006 (Annex) the use of the claim "SOURCE OF OMEGA-3 FATTY ACIDS" is restricted. The Annex states:

"*A claim that a food is a source of omega-3 fatty acids, and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 0,3 g alpha-linolenic acid per 100 g and per 100 kcal, or at least 40 mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal*". This is clearly not the case for MON 87769 – which contains enhanced SDA levels intended to alter omega-3 levels via the metabolism of the consumer - and therefore the implication for the consumer that the product contains omega3 fatty acids should be avoided. For all three soybeans, the altered nutrient levels were introduced with the objective for the applicants of making claims of health benefits, yet no such applications have been made under Regulation (EC) No. 1924/2006. Since the GMO assessment process considers only risks, it is not the right mechanism to approve or imply health claims for labels.

Although not currently provided for in the legislation, labelling of meat, milk and dairy products from animals fed on nutrient-altered soybeans 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.

Lack of Guidance for the assessment of nutritionally-altered crops has led to a situation where the labelling requirements from such crops which are necessary to protect human health have not been developed. This has led to inconsistent and inadequate labelling proposals for the three soybean products which fail to meet the requirements of Regulation 1829/2003.

1.2.8 Post-market monitoring

Commission Implementing Regulation (EU) No. 503/2013 notes in Recital (19) that it is appropriate to confirm the expected consumption, application of conditions of use or identified effects via post-market monitoring in cases where the GM food or feed has altered nutritional composition.

The proposed post-market monitoring (PMM) plans for all three products are inadequate to identify unintended health effects:

- For MON 87769, the Commission Implementing Decision states that: "*1. The authorisation*

holder shall collect the following information: (i) quantities of MON-87769-7 soybean oil and MON-87769-7 soybeans for oil extraction, imported into the European Union for the placing on the market as or in products for food; (ii) in case of import of products referred to in point (i), results of searches in the FAOSTAT database on the quantities of vegetable oil consumption by Member State, including shifts in quantities between the different types of oils consumed; (iii) in case of import of products referred to in point (i), data on the different categories of food and feed uses of MON-87769-7 oil in the EU; and 2. The authorisation holder shall, based on the information collected and reported, review the nutritional assessment conducted as part of the risk assessment".

- For MON 87705, the Commission Implementing Decision states that: "*1. The authorisation holder shall collect the following information: (i) quantities of MON-877Ø5-6 soybean oil and MON-877Ø5-6 soybeans for oil extraction, imported into the European Union for the placing on the market as or in products for food; (ii) in case of import of products mentioned under (i), results of database searches in the FAOSTAT database on the quantities of vegetable oil consumption by Member State, including shifts in quantities between the different types of oils consumed. 2. The authorisation holder shall, based on the information collected and reported, review the nutritional assessment conducted as part of the risk assessment*".
- For soybean 305423, the Commission Implementing Decision states that: "*1. The authorisation holder shall collect the following information: (i) quantities of DP-3Ø5423-1 soybean oil and 305423 soybeans for oil extraction, imported into the European Union for the placing on the market as or in products for food; (ii) in case of import of products mentioned under (i), results of database searches in FAOSTAT database on the quantities of vegetable oil consumption by Member State, including shifts in quantities between the different types of oils consumed; and 2. The authorisation holder shall, based on the information collected and reported, review the nutritional assessment conducted as part of the risk assessment*."

However, as noted in Section 2.2 above, data on expected consumption has been based largely on UK data for all three soybean applications. As stated in Regulation 503/2013 Recital (19), it is not appropriate to delay consideration of consumption data elsewhere in the EU to the post-market monitoring stage. Further, consumption data should also have been collected for all vulnerable groups prior to approval (as discussed in Section 1.2.4).

In addition, the failure to properly assess potential health impacts prior to authorisation (as discussed in Section 2) makes it impossible for PMM to fulfil the role of monitoring such effects. This is because prior hypotheses for adverse effects (such as potential effects on cancer risk) need to be formulated before the product is approved if a meaningful monitoring regime is to be implemented.

According to Codex (Codex Alimentarius, 2003):
"*Post-market monitoring may be undertaken for the purpose of:*

A) verifying conclusions about the absence or the possible occurrence, impact and significance of potential consumer health effects; and

B) *monitoring changes in nutrient intake levels, associated with the introduction of foods likely to significantly alter nutritional status, to determine their human health impact*."

It is not possible for PMM to fulfil this role if intakes of relevant nutrient levels throughout the EU have not been established in the applications and if potential adverse health effects associated with changes in fatty acid levels raised in the scientific literature have not been considered (see Section

1.2.1 above).

Article 7 of Regulation 503/2003 specifies some requirements for post-market monitoring. Under Article 7.1, PMM is required when it is appropriate to confirm:
"(a) that specific recommendations of uses are followed by the consumer/animal owner;
(b) the predicted consumption of the genetically modified food or feed; or
(c) the relevance and intensity of effects and unintended effects detected during the pre-market risk assessment which can only be further characterised by post-market monitoring".
Article 7.2 specifies that the applicant shall ensure that the post-market monitoring is:
"*(a) developed to collect reliable information with respect to one or several of the aspects set out in paragraph 1. This information shall allow the detection of indications on whether any (adverse) effect on health may be related to genetically modified food or feed consumption;*
(b) based on strategies aiming at collecting relevant information from specific stakeholders including consumers and on a reliable and validated flow of information between the different stakeholders. More specific strategies shall be included when data on individual intakes of a specific food item or intakes of particular age groups have to be collected;
(c) accompanied by adequate justification and a thorough description of the selected methodologies for the proposed post-market monitoring including aspects related to the analysis of the collected information."

Again, it is not possible for PMM to fulfil these roles if intakes of relevant nutrient levels throughout the EU have not been established in the applications and if potential adverse health effects raised in the scientific literature have not been considered (see Section 1.2.1 above). In particular, there is no proposed collection of information to allow the detection of indications on whether any (adverse) effect on health may be related to genetically modified food or feed consumption, or to collect data from particular age groups.

Failure to develop Guidance for the assessment of nutrient-altered crops means the proposed monitoring plans for all three soybeans are inadequate.

1.2.9 Summary

In summary, the lack of specific guidance for nutrient-altered GM crops has led to inconsistent and inadequate risk assessments for all three oil-altered GM crops considered by EFSA to date. Issues include:

- The GM traits all affect multiple nutrients and the overall effect on health is poorly understood: health claims (of benefits) are not substantiated;
- Because, unlike previous GM crops, nutritionally-altered GM crops are engineered to produce molecules that are biologically active in humans, there is an increase in the risk of adverse medical effects either from over exposure to the intended product or from unintended by-products whose hazard to health is unknown;
- Gene-environment interactions will affect nutrient expression and the field trials conducted are inadequate to characterise the resulting variability in nutrient levels;
- There has been no full nutritional/food safety analysis (instead the focus is on comparing the main altered nutrient with standard dietary recommendations);
- Potentially vulnerable subgroups need to be considered;
- Impacts of food processing and storage need to be considered for all food types;
- Use of the GM crop as feed can alter nutrient content of (unlabelled) meat and dairy products;
- Food labelling proposals are inadequate to provide sufficient information for consumers;
    - Post-market monitoring is inadequate to identify adverse health effects.

2. Implications of lack of full nutritional/food safety assessment for other future nutritionally-altered crops

Other nutritionally altered crops may in future contain altered levels of vitamins and minerals (Garcia-Casal et al., 2016). Thus far, biofortified products introduced in Latin America, Africa, and Asia have been produced only by conventional breeding: traits include enhanced pro-vitamin A (in banana, cassava, sweet potato, pumpkin and maize), and iron and/or zinc (in cereals or legumes) (Mejia et al., 2017). However, many genetically engineered crops with altered nutrients are at the experimental stage (De Steur et al., 2015). Some examples, and the food safety issues that they raise, are discussed below.

2.1 Beta-carotene

The enhanced beta-carotene levels in GM ‘Golden Rice’ are intended to be metabolised to vitamin A in the human body, combating vitamin A deficiency. Claims that GM 'Golden Rice' would bring major benefits to children in the developing world were first made in the press in 1999. GM 'Golden Rice' appeared on the front page of Time magazine in August 2000, with the headline “ This rice could save a million kids a year ” (TIME, 2000), as well as in numerous press articles around the world. A deal with AstraZeneca (now Syngenta) was announced to offer vitamin-A-rich GM rice free to farmers in the developing world to combat blindness (The Golden Rice Project, 2005). The claims of health benefits from GM Golden Rice remain controversial and it has not yet been commercialised. When the biotechnology industry first began promoting GM rice as a means to prevent blindness in poor children, the levels of beta-carotene in it were too low to contribute significantly to tackling vitamin A deficiency. A second generation of Golden Rice (GR2) was later developed which contains much higher levels of beta-carotene (Paine et al., 2005). As shown by Testbiotech (Then & Bauer-Panskus, 2018) in regard to the nutritional quality of GR2, the potential benefits of the rice as, for example, claimed by Paine et al. (2005) are greatly overestimated and cannot be realised under practical conditions. At the same time, it is evident that substantial losses have of carotenoids have to be expected. Schaub et al (2017) show that quick degradation can be expected from storage of GR2 rice grains; this is in addition to the losses from parboiling.

Furthermore, claims that Golden Rice is a cost-effective means to reduce vitamin A deficiency rest on unverified assumptions about bioavailability of vitamin A from the rice and the dose-response curve (Haskell, 2012; Krawinkel, 2007, 2009; Nestle, 2001; Tang et al., 2012). It remains unclear whether beta-carotene-fortified crops can improve vitamin A status in the main targets of the biofortification efforts, that is, malnourished adults and children (Giuliano, 2017a). For example, one study found that beta-carotene biofortified maize (produced through conventional breeding) did not increase retinol (vitamin A) in marginally malnourished children (Palmer et al., 2016b); and the maize also had limited impact on breast milk retinol concentrations (Palmer et al., 2016a). In contrast, if very high levels of beta-carotene are ever achieved, then there is concern that individuals may consume too much, potentially resulting in toxicity and/or an increased cancer risk (see below).

Altering the beta-carotene pathway in rice through genetic engineering has the potential to produce other biologically active retinoids, which could have adverse effects on human health or development (Schubert, 2008). Retinoids are a family of compounds derived from plant carotenoids that are required for many aspects of human health and development, but which may also be toxic

or teratogenic (damaging to embryo development). Because of the complexity of the relevant biochemical pathways, the transgenic lines may induce hitherto undiscovered feedback mechanisms with unpredictable results: there are still numerous unknown aspects of carotenoid biosynthesis in staple cereals (Zhai et al., 2016). Genetic engineering of the carotenoid pathway has been shown in several cases to alter the development and metabolism of the resulting GM plants, through unintended changes to non-carotenoid metabolism (Giuliano, 2017b). Recently published evidence of yield problems with Golden Rice as a result of these unintended effects are discussed further in Section 3.4.

Other studies have revealed that overproduction of beta-carotene can cause changes in plant phenotypes, fatty acid compositions, and protein content. For example, a study of beta-carotene-enhanced soybean lines has highlighted significant differences in levels of crude fat, carbohydrate, δ-tocopherol, and oleic acid of transgenic soybeans compared to those of non-transgenic counterparts, which could be due to the influence of the transgene insertion (Qin et al., 2017). The authors argue that alterations to composition require further studies, such as transcriptome and metabolome profiling.

The intellectual property (IP) associated with Golden Rice was only donated to poor farmers after two major clinical trials (published in 1994 and 1996) found that high doses of beta-carotene, which had been believed to reduce cancer risk as an anti-oxidant, unexpectedly increased the risk of cancer in smokers and asbestos workers (Duffield-Lillico and Begg, 2004; Goodman et al., 2004; Omenn et al., 1996; Omenn, 2007; The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994). As a result, companies' plans to market beta-carotene-enhanced GM foods to wealthy consumers as an anti-oxidant "functional food" – intended to reduce the risk of cancer – were abandoned (Watkins et al., 2001). The authors of this paper state "*Such unexpected responses among specific populations exemplify why the food and agriculture industries cannot afford to target improvements in health with single modifications in food composition based on conclusions inferred from the measurement of single biomarkers*".

It is unclear whether the increased cancer risk associated with high doses of beta-carotene is restricted to smokers, however some animal studies suggest this may be the case (Russell, 2004). If so, there may be a particular issue for those exposed to smoke from cooking stoves in the target countries for growing Golden Rice, whereas in EU countries and Switzerland, smokers may be at highest risk. Risk assessments of beta-carotene enhanced GM crops will need to consider these vulnerable subgroups. Importantly, adverse effects such as these can only be identified by long-term clinical studies in humans. Further, even large-scale, long-term trials have often failed to provide definitive answers about the benefits and risks of high doses of nutrients in relation to endpoints such as cancer (Kotecha et al., 2016).

2.2 *Iron and zinc*

Unlike beta-carotene and many other nutrients, minerals such as iron and zinc cannot be synthesised in plants. Efforts to increase mineral levels in plants have therefore focused on increasing uptake from the soil, or concentration of minerals in the edible parts of the plant, or on reducing anti-nutrients such as phytates which inhibit the bioavailability of iron.

One problem with attempts to genetically engineer enhanced iron and zinc content in plants is that such plants may also accumulate toxic metals such as cadmium if they are planted in contaminated soils (Nakandalage et al., 2016; Palmgren et al., 2008; Slamet-Loedin et al., 2015; Zhang et al., 2012; Zhao and McGrath, 2009). This is an example where it is critical to assess the risk of gene-

environment interactions, not only on the dose of the intended nutrient but also on the dose of other substances.

As for many other nutrients, iron supplementation requires a careful balance between the benefits in tackling iron deficiencies and the health risks of excess iron: including adverse effects on gut microbiota, leading to diarrhoea, and possible increased risks of other diseases (Prentice et al., 2017). Additional intake of iron can also favour malaria. Further, the risks to subgroups at particular risk from increased iron intake must be assessed, including those with hemochromatosis and the common genetic disorder Beta thalassemia.

3. Environmental impacts of potential future cultivation applications

No applications for commercial cultivation of nutritionally-altered GM crops have been made to date in the EU or Switzerland. However, there are examples of unintended effects from experiments. The lack of predictability of adverse effects on wildlife limits the applicability of environmental risk assessments.

3.1 *Direct adverse effects on wildlife of consumption of altered nutrients*

One study found that the presence of the omega-3 fatty acids EPA and DHA in diets of larval small white butterflies (*Pieris rapae*) may result in progressively heavier adults, with smaller wings and a higher frequency of wing deformities (Hixson et al., 2016). Although this is a single study with some limitations, it raises concerns regarding potential direct adverse effects on wildlife from the consumption of altered nutrients in GM crops. A lot more research would be needed to assess the risk of similar impacts on all relevant species for all nutritionally-altered GM crops, before cultivation authorisations could be granted. In particular, the study highlights that what is perceived to be a nutritional compound for one species, e.g. humans, can be harmful to other species which may inadvertently ingest the plant.

3.2 *Complex ecological effects*

Production of the omega-3 long-chain fatty acids EPA and DHA by GM-oilseeds has been introduced experimentally in oil seed rape (canola) and camelina. A recent review has highlighted that the novel production of these fatty acids in crops may result in unintended ecological and evolutionary effects (Colombo et al., 2018). EPA and DHA are currently naturally produced, primarily by algae, in aquatic environments, but are not known to be produced by terrestrial crops. The paper notes that the production of EPA and DHA by GM seed oil crops represents a fundamental shift in the accessibility of bioactive fatty acids to terrestrial consumers of these crops; one that may change their physiology and survival thereby altering ecological interactions among terrestrial organisms. The paper cites evidence that production of EPA and DHA by terrestrial crops has the potential to impact not only primary consumers (e.g., herbivorous insects, rodents, birds), but also their secondary (e.g., insectivorous birds, bats, and insects etc.), and tertiary consumers (e.g., foxes, predatory birds), affecting growth rates and performance. For example, small mammals (e.g., rodents, rabbits) that may consume the seeds directly could enjoy a competitive advantage in consuming these crops, in having a direct source of EPA and DHA in their diet. The impact that EPA and DHA may have on the biology of terrestrial invertebrates is poorly understood. If the same

effects of EPA and DHA occur in terrestrial invertebrates as are observed in aquatic invertebrates, then the growth rate, reproductive success, and/or survival of crop pests may increase. In addition, gene flow may occur between GM crops and wild relatives. Thus, dispersal and persistence of EPA and DHA in the terrestrial environment could have biochemical, ecological, and evolutionary effects that may be irreversible.

The paper argues that the large-scale, novel, introduction of these two highly bioactive fatty acids into the agro-ecosystem may have unintended, cascading, effects throughout terrestrial food webs. The authors advocate careful regulatory consideration of these new GM-oilseed crops because these highly bioactive fatty acids could be consumed and metabolized, for the first time, by animals in the agro-ecosystem. For regulatory purposes, specific testing of their potential ecological effects is therefore warranted.

3.3 *Increased attractiveness to pests*

One potential negative impact of increasing the nutrient content of plants is that they may become more attractive to some pests, since enhanced nutrient content will tend to increase the plant's nutritional quality for anything feeding on the plant (Branco et al., 2010). Attractiveness to pests may also be increased by particular approaches to increasing nutrients in plants: for example, one way to increase calcium content in plants is to reduce calcium 'anti-nutrients' such as oxalate or phytate. However, oxalate is part of the plant's defence system against pests, so reducing it could increase the palatability of the plant to pests (Dayod et al., 2010).

There is a lack of experimental evidence regarding the risk of increasing attractiveness to pests, which currently makes comprehensive environmental risk assessments difficult.

3.4 *Susceptibility to pathogens*

In one experiment, an attempt to make GM soybeans with improved fatty acid content also showed a marked increase in the accumulation of Bean pod mottle virus and enhanced susceptibility to the bacteria Pseudomonas syringae, which attacks a wide range of plants (Singh et al., 2011). These effects are not fully understood but are likely to be related to unintended changes in the chemical defences of the plant. There is a lack of experimental evidence regarding how altered nutrients could affect susceptibility to plant pathogens, which currently makes comprehensive environmental risk assessments difficult.

3.5 *Adverse impacts on yield and agronomic properties*

Backcross derived lines of GM Golden Rice in the genetic background of a mega rice variety called Swarna showed abnormalities in the growth and development of the lines (Bollinedi et al., 2017). The plants were shorter in height and there was a drastic reduction in yield, as well as other changes. The total chlorophyll content of the GM plants was also significantly lowered. Similar observations were also made in the original transgenic American long grain rice (Kaybonnet) plants. The authors conclude that insertion of the transgene had disrupted another gene which plays a role in regulating the root growth of the plant. They also show that changes in the pathway for β-carotene production affected other biochemical pathways, which may have affected growth. They conclude that, due to the complexity of interactions among various plant hormones, it is difficult to demonstrate the corresponding magnitude of these two adverse effects.

In another example, tomato plants genetically engineered to have increased carotenoid levels also

showed a reduction in height (dwarfism) (Fray et al., 1995). This unintentional effect is thought to be due to the intentional over production of phytoene synthase leading to competition for geranylgeranyl diphosphate (GGDP) between the gibberellin (GA), phytol and carotenoid biosynthetic pathways. Changing the same pathway in Arabidopsis showed increased levels of chlorophyll and abscisic acid, which led to delayed seed germination and seed dormancy (Lindgren et al., 2003).

These two types of problems (unintentional gene disruption and unexpected changes to biochemical pathways) could occur in any nutrient-altered GM crop, and highlight the need for extensive studies to characterise the unintended impacts of genetic changes on agronomic properties.

3.6 *Other altered properties with potential ecosystem impacts*

In the Golden Rice experiments discussed above, the altered properties of the GM rice included not only reduced height and yield but also delayed heading (by nine days), incomplete panicle emergence and smaller panicle size, two times more tillers, atypical nodal branching, and reduced spikelet fertility (Bollinedi et al., 2017). Other observations included increased leaf number, pale green leaves and reduced flag leaf length. One experiment with rice genetically modified to increase iron content also led to earlier flowering in these plants (Wirth et al., 2009). Altered flowering times could potentially have adverse effects on pollinators and hence on the crop and wider ecosystem.

These observations highlight the complexity of the potential unintended effects of altered biochemistry on the properties of plants. Potential adverse effects on ecosystems of these altered properties need to be included in any risk assessment of these plants.

3.7 *Contamination of non-GM crops or wild relatives*

Cases of contamination of non-GM crops by GM crops have been reported worldwide (Price and Cotter, 2014). For some nutrient-altered GM crops contamination issues could be particularly significant e.g. with omega-3 altered oil seed rape being proposed for growing on an industrial scale for use as fish feed. Oil seed rape grows readily as a feral plant and therefore the GM trait can spread widely in the environment (Schulze et al., 2014).

In some cases nutritionally-altered plants may have enhanced fitness, with additional implications for their likely spread and impacts on biodiversity (as discussed in the Enhanced Fitness subreport).

4. Conclusions

In contrast to most GM crops currently on the market, nutritionally-altered GM crops are genetically engineered to produce small molecules that are biologically active in humans. It is therefore particularly important to assess the impacts of these products on human health.

Such crops are not regarded as “substantially equivalent” to existing conventional crops and therefore pose new challenges for regulators. Nutritional changes are complex and not limited to a single nutrient. There is a lack of scientific consensus on the impacts of altered nutritional content on human health and this may vary with dose (which is in turn highly dependent on the environmental conditions where the plant is grown) and the target population. Some subpopulations may be put at risk if the nutrient content of food is altered, even if the food is safe for other people in a given population. Due to the complexity of nutritional changes that can be introduced through genetic engineering, adequate labelling is challenging.

There is ample evidence of potential unintended effects of altered biochemistry on the properties of nutritionally-altered GM plants, which may occur due to (i) unintended disruption of other genes during the process of insertion of the transgene, and/or (ii) the unintended consequences of altering complex biological pathways. Intentional and unintentional changes in nutritional content may impact nutritional content; potential toxicity or sublethal effects in humans and wildlife; complex ecosystem properties; agronomic properties of plants; and/or the susceptibility of plants to pests and pathogens.

The stacking of other traits (herbicide tolerance) and the use of RNA interference in some products add to the complexity of any risk assessment. Relevant issues are considered in other subreports.

The lack of Guidance for the assessment of nutritionally-altered GM crops has resulted in an inadequate assessment process which fails to protect human health. There are also major inconsistencies between the information supplied for different products. Specific guidance for the risk assessment of nutritionally-altered GM crops should have been adopted by EFSA before any imports for food and feed were approved, and there should have been full public consultation on such guidance. Specific guidance will be of even greater importance before undertaking environmental risk assessments for nutritionally-altered GM crops.

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