GE Free New Zealand

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23/8/2013

Application A1081
Food derived from Herbicide-tolerant Soybean Line SYHT0H2

We would like you reject this application, option 2.

Due to the lack of feeding test and changes to the nutritional components of the corn it is not possible to evaluate this application A1081 for human safety ingestion.

Until FSANZ has implemented the EFSA standards on GE food assessments, namely long term feeding studies, and assessing compositional differences, FSANZ is subjecting the peoples of Australia and New Zealand to a substandard assessment regime that is not based on your science guarantees.

We are notably concerned at the lack of scientific analysis that has been conducted on mammals in support of this application, A1081. There is no detailed critique of any cross referencing to other studies due to the lack of vital information on how the new transgenic food will affect human consumers.

We are entering this dialogue as part of the consumer consultation process as outlined in The FSANZ Application Handbook and as part of a major variation of a genetically engineered (GE) food application, namely A1081. We note that

> *FSANZ needs to ensure that it has collected sufficient evidence, including from outside experts if necessary, in order to be able to undertake a rigorous analysis of each case. In some situations the best available scientific evidence is irrefutable. In others there might be conflicting scientific views, a lack of evidence or some uncertainty in the science. Where the evidence is in dispute, FSANZ will ensure that it sets out the reasoning and logic used to reach its decision/s.*[1]

The FSANZ Science Strategy 2012 -2015 talks about data gathering, peer reviewed science and looks at enhancing "our" science by a risk analysis that is evidence and outcome based.

> *FSANZ ensures that food regulatory measures are based on the best available scientific evidence, using a risk analysis framework. The successful application of science is critical to the effectiveness and appropriateness of food regulatory measures, and underpins the risk*

[1] Community involvement and consultation during the assessment process http://www.foodstandards.govt.nz/foodstandards/changingthecode/informationforapplicants/communityinvolvement3610.cfm

management decision-making process.[2]

It is concerning that in every FSANZ assessment application for a GE food the public is led to believe that the experts at FSANZ are assessing the safety of eating these products on hard scientific evidence from outside experts as well as industry.

Of note, the same statement made in this assessment appeared in the last two FSANZ statements when assessing risk of GE foods -

> *The Safety Assessment did not identify any public health and safety concerns associated with the genetic modification used to produce...*

This leads the public to assume that safety studies have been conducted on either human or mammals. Yet when asked for this scientific information for the results of the whole GE plant being fed to mammals or humans **it has not been done**. The evidence simply does not exist. There is no scientific data to support such statements though FSANZ states in the FSANZ Application Handbook and the FSANZ Science Strategy 2012 -2015 require it.

FSANZ has the discretion to "stop the clock" to ask for more information[3]. The current application for A1073 is devoid of any scientific analysis in relation to ingestion safety and must be immediately put on hold until sufficient safety feeding data is obtained.

FSANZ is charged with protecting human health in Australasia in relation to section 18 (b) to protect human or animal life or health from risks arising from additives, contaminants, toxins or disease-causing organisms in foods, beverages or feedstuffs.

We believe that under section 50 (i) (ii)(iii) of the FSANZ Act, when the application for A1081 was considered, the following requisite information (p.38) was not available:

- the initial requirements to the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices;
- the prevention of misleading or deceptive conduct; [4]

This is a major variation of legal requirements[5] with no preceding safety studies conducted on the

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[2] Our Science www.foodstandards.govt.nz/scienceandeducation/scienceinfsanz/

[3] FSANZ will have the discretion to 'stop the clock' for up to 18 months for Applications if the Ministerial Council has notified FSANZ that it is developing policy guidelines on a specific, clearly defined issue or subject matter.
http://www.foodstandards.govt.nz/scienceandeducation/publications/annualreport/fsanz annualreport20062007/ourregulatorymeasures/newproceduresforamen3670.cfm

[4] Food Standards Australia New Zealand Act 1991 Act No. 118 of 1991 as amended

[5] *Major procedure (12 months to complete assessment)* Applies to the development of a new food standard or a major variation to a food regulatory measure involving considerable scientific or technical complexity.

soybean product in question. We believe that FSANZ needs to maintain its duty to protect public health.

Adequate information needs to be made available to submitters to make informed submissions in this democratic process of stakeholder engagement. We are specifically seeking scientific data on the biological effects of eating the proposed food product.

As FSANZ is the first of the member Food Authorities of Codex to receive and assess this application there needs to be a careful review of the scientific evidence in support of the application.

There does not seem to be any heed taken to the Codex guidelines as set out below in relation to this as yet untested and unapproved food, that has not even been approved for commercial planting.

It is of great concern that in the rush to approve this transgenic Soybean for commercialisation, environmental and ingestion studies have not been completed.

As the consumer information on FSANZ website says

> *Where possible, submissions should contain scientific evidence rather than conjecture to back up any assertions as FSANZ is required to use the best scientific evidence available in its decision-making processes.*[6]

Yet FSANZ, an expert government food assessment body has requested the public to provide informed submissions on something without any data.

We would like to reiterate that there has been no scientific information on the most important part of risk assessment –feeding data on any potentially adverse effects that might arise out of eating this soybean. In this regard, FSANZ has not followed its own protocols. We would like to submit our review of valid, peer-reviewed scientific information from publications that demand consideration above the industry assurance of safety. It is not acceptable that FSANZ requires scientific data from submitters yet its experts rely on Industry assurances without any scientific proof.

This is especially relevant due to the comprehensive feeding trials (Seralini, 2012) that were published in the Journal Food and Chemical Toxicity 2012 documenting the lifetime feeding of rats and the severe adverse effects and deaths that were recorded in the rats fed transgenic product, those animals developed serious health problems in the livers, kidneys and developed endocrine related testicular, uterine and mammary tumours leading to euthanasia or death while controls remained healthy.[7]

Tolerance to glufosinate ammonium expressed by the enzyme *phosphinothricin acetyltransferase* (PAT) obtained from the soil bacterium *Streptomyces viridochromogenes* and tolerance to Mesotrione through

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[6] Information for Submitters
http://www.foodstandards.govt.nz/foodstandards/changingthecode/informationforsubmit 1129.cfm

[7] Seralini. G-E., Clair. E., Mesnage. R., Gress. S., Defarge. N., Malatesta. M,. Hennequin. D. and de Vendomois. JS. (2012) Long term toxicity of a Roundup herbicide and a Roundup- tolerant genetically modified maize. Food and Chemical Toxicity. Vol: 50, (11) 4221– 4231
http://dx.doi.org/10.1016/j.fct.2012.08.005

expression of the AvHPPD -03 protein encoded by the avhppd-03 gene from A*vena sativa* , these are untested gene combinations, and new food that has never entered the food chain before. Unless approval is halted for until proper scientific evidence can demonstrate its safety, further action will be considered. We are writing this noting that many of the references FSANZ is guided by for food safety are taken from the OECD and EFSA guidelines on GM Foods. We presume that the harmonization and default position is to these bodies when assessing food safety. We are of the understanding that feeding studies are to be part of the review process in major applications.

Does FSANZ follow the OECD guidelines for GE foods safety? If so, the FSANZ Act requires that a major new application such as that for SYHT0H2 has a proper scientific assessment.

For submitters, the most crucial information about the new product is how it will affect them when they eat it, and what adverse effects people can expect from eating the whole food. Consumers cannot make informed comment when the information that would affect their health is unavailable. The assessment report provided is highly misleading since there is in fact, no data on the health effects of eating this whole soybean food.

GE Free NZ would like this application to be immediately recalled and the clock stopped until feeding studies are conducted. This is an untested and potentially dangerous food with significant public health implications. We outline our concerns in this matter below:

1. **Compositional equivalence – There are many concerns about the evaluation that FSANZ has made. "Compositional equivalence" that is similar to "substantial equivalence" and is a new term (biological significance) not seen before and we do not know what it means. Please could you clarify? Specifically, if there are no feeding studies conducted on mammals /humans how did FSANZ deduce that there were no effects of "biological significance"?**

2. **Alteration of nutritional parameters**

The fact that this application event SYHT0H2 contains novel genes to enable the soy plant to withstand three different herbicides in itself makes the soybean compositional equivalence different from its non transgenic control. To refresh the reviewer's memory of this fact, we outline the relevant places where the significant differences are detailed in the Dow compositional analysis report.

Proximate and Fiber Analysis of Seed

Statistically significant overall treatment effects were found

Mineral Analysis of Seed

Statistically significant differences were observed for SYHT0H2

Amino Acid Analysis of Seed

Statistically significant differences were observed

Bioactive Analysis of Seed

Statistically significant differences were observed for lectin, raffinose, trypsin inhibitor, entries compared with the control.

These statistical differences between the control and the soybean SYHT0H2 event means that under OECD, 2003; EFSA, 2008 long term mammal feeding tests are triggered and must be conducted on the whole soybean.

3. **OECD, Codex Alimentarius, EFSA. The three transgenic proteins in soybean** SYHT0H2 **have never been used in combination before, nor have the levels of herbicide applied been used in the growing of these foods. Under the EFSA and OECD guidelines, this constitutes a need to demonstrate that these newly expressed proteins and accompanied herbicide residue should undergo animal feeding studies to show they will not adversely affect human health. According to the European Food Safety Authority in 2011[8] the applicant should provide in relation to the safety of newly expressed proteins,**

   e) repeated dose toxicity studies using laboratory animals, unless reliable information demonstrating the safety of the newly expressed protein (including its mode of action) can be provided, and it is demonstrated that the protein is not structurally and functionally related to proteins adversely affecting human or animal health. The repeated dose 28-day oral toxicity study in rodents with the newly expressed protein should be performed according to OECD guideline 407 (Table 2). It is recommended to use a sufficient number of animals per group e.g. 10/sex in order to obtain an adequate statistical power. Depending on the outcome of the 28-day toxicity study, further targeted investigations may be required.

Under clause 3.3.2 of the EFSA guidance for risk assessment for food from GM plants, toxicological assessment tests should be conducted to show any toxicological effects such as

- dose response relationships
- threshold levels
- delayed onset of adverse effects
- risks for certain groups in the population
- use of uncertainty factors in extrapolating from animal data to humans It is known that soybean has naturally occurring toxins. Relative concentrations of these could be altered by the engineered event. Though temperature studies have been conducted there is no data to elucidate whether the intact DNA survived heating, or if the foreign DNA could pose a more significant immunological reaction if it enters the blood stream.

Until scientific feeding tests are conducted, none of these major effects can be assessed. The following clause 3.3.3 states that "the applicant should ensure that the final risk characterisation clearly demonstrates that:

a) consumption of food and feed derived from GM plants is as safe as the respective comparators;

[8] EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011; 9(5): 2150. [37 pp.] doi:10.2903/j.efsa.2011.2150. Available online: www.efsa.europa.eu/efsajournal.htm

b) the food derived from a GM plant is not nutritionally disadvantageous for the consumer compared to the food which is intended to replace;

c) the feed derived from a GM plant feed is not nutritionally disadvantageous for animals compared to the feed which is intended to replace;

d) the feed derived from a GM plant does not harm or mislead the consumer by impairing distinctive features of the animal products compared to conventionally produced feed.[9]

The applicant has not been able to demonstrate that the SYHT0H2 event is safe in respect to any of these points as there are no feeding studies to look at adverse effects.

This statement contradicts the Codex Alimentarius Foods derived from modern biotechnology on unintended effects have not been considered that states-

*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. (*point 15 & 16, p.2)[10]

As reported on the Bioactive analysis of seed showing a significant difference between anti nutrient, Codex says

In the case of proteins, the assessment of potential toxicity should focus on amino acid sequence similarity between the protein and known protein toxins and anti-nutrients (e.g. protease inhibitors, lectins) as well as stability to heat or processing and to degradation in appropriate representative gastric and intestinal model systems. Appropriate oral toxicity studies may need to be carried out in cases where the protein present in the food is not similar to proteins that have previously been consumed safely in food, and taking into account its biological function in the plant where known. (point 38, p.6)

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 bio-availabilities of nutrients are expected or if the composition is not comparable to conventional foods. In addition, 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. (Codex Alimentarius, p.17)

Potential accumulation of substances significant to human health

54 Some recombinant-DNA plants may exhibit traits (e.g. herbicide tolerance) that may indirectly result in the potential for accumulation of pesticide residues, altered metabolites of such residues,

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[9] Guidance for risk assessment of food and feed from genetically modified plants. EFSA Panel on Genetically Modified Organisms (GMO) European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2011; 9(5):2150 .

[10] Guideline For The Conduct Of Food Safety Assessment Of Foods Derived From Recombinant-Dna Plants *CAC/GL 45-2003* *http://ec.europa.eu/food/food/biotechnology/qanda/i2_en.print.htm*

toxic metabolites, contaminants, or other substances that may be relevant to human health. The safety assessment should take this potential for accumulation into account. Conventional procedures for establishing the safety of such compounds (e.g. procedures for assessing the human safety of chemicals) should be applied. (Codex Alimentarius p.18)

Significant differences were shown between the control soybean and the GE soybean SYHT0H2. These differences are expressed by the novel genes inserted into the plant DNA (which have never been in food before) and the possible interaction between the product-required herbicides used to produce the soybean as well as the metabolites produced by the recombinant DNA in transgenic soybean plants.

In accordance with Codex Alimentarius 2009, animal feeding studies must be conducted with adequate immunoresponse testing to ensure that the allergenic potential of the immunoreactive proteins in both cooked and uncooked soy SYHT0H2 do not cause allergic reactions in laboratory animals (Section 4 - Codex Alimentarius, p.22). We also consider that the bioavailability of nutrients, introduced foreign proteins and foreign DNA be assessed and the significance of nutrient alteration is tested, as significant compositional changes in soybean SYHT0H2 have been demonstrated (Section3 Codex Alimentarius, p.25).

Summary –

1. The new food soybean SYHT0H2 has not been found safe for human

2. This application does not follow Codex Alimentarius protocols on GE food assessments.

3. FSANZ has misled the public in its finding on SYHT0H2 safety for public consumption

4. This application has disregarded the statistical differences between the composition of GE food SYHT0H2 and “Jack” soybeans

5. This application must be put on stop until long term feeding studies are conducted and independently assessed.

Yours sincerely,

Jon Muller

Secretary GE Free NZ in Food and Environment.

GenØk - Centre for Biosafety

Vår ref:2013/h111
Deres ref: 2013/1392 ART-BI-DHT

Direktoratet for naturforvaltning
Tungasletta 2
7485 Trondheim
Dato: 11.03.2013

Vedlagt er inspill fra GenØk – Senter for Biosikkerhet om høringer **EFSA/GMO/NL/2012/111** for SYHT0H2 soya fra Syngenta Crop Protection AG

Vennligst ta kontakt hvis du har noe spørsmål.

Med vennlig hilsen,

Lise Nordgård
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KONKLUSJON PÅ NORSK
Vi trekker frem mangler i dossieret som ikke gir grunnlag for en konklusjon om sikker bruk, samfunnsnytten og bidrag til bærekraftighet av SYHT0H2 soya fra Syngenta Crop Protection AG. Søker har ikke inkludert noe av den informasjonen omkring samfunnsnytten og bærekraftighet til SYHT0H2 soya som kreves i den norske genteknologiloven (Appendix 4) for godkjenning i Norge.

Hovedkonklusjon og anbefalinger
Genøk – Senter for Biosikkerhet viser til brev fra Direktoratet for naturforvaltning (DN) angående høring som omfatter SYHT0H2 soya for bruksområdene import, prosessering, mat og fòr.

Soyaplanten SYHT0H2, er en stablet hybrid med ulike herbicid-kodende gener innebygd. Stablede hybridplanter har generelt en mer kompleks genetisk sammensetning og derfor større potensiale for opp- og nedregulering av plantens egne gener. En grundig testing før evt markedsadgang vil derfor være nødvendig. Søker bør fremskaffe eksperimentelle bevis som viser at kombinasjonen ikke er skadelig og ikke bare vise til antagelser basert på vurderinger gjort av disse proteinene hver for seg.

I tillegg er plantevermidlet glyfosat-ammonium som SYHT0H2 bl.a.er genmodifisert til å gi plantene resistens mot, ikke lovlig i Norge eller EU (med unntak av begrenset bruk på epler). Vi mener en godkjennelse av SYHT0H2 vil skade grunnleggende etiske og sosiale kriterier for bruk, som omtalt i den norske Bioteknologiloven.

Søker bør på bakgrunn av den nylig publiserte artikkelen av Podevin og du Jardin med tittelen; “Possible consequences of the overlap between the CaMV 35S promoter regions in plant transformation vectors used and the viral gene VI in transgenic plants”, utvide den molekylære karakteriseringen av denne eventen og se på muligheten for ulike RNA varianter, fusjonsproteiner og del utrykk av P6. Artikkelen har ført til en diskusjon om tidligere godkjenninger av GM-planter har oversett kritiske sikkerhetsspørsmål knyttet til bruken av Cauliflower mosaic virus 35S promotor (P35S) i GM-planter.

Søker gir ikke opplysninger som adresserer vurderingskriteriene bærekraft, samfunnsnytte og etiske aspekter som forutsettes anvendt i den norske genteknologiloven. I denne sammenheng er det viktig å få dokumentert erfaringer med hensyn på effekter på miljø, helse og samfunnsaspekter. Denne type dokumentasjon er ikke vedlagt søknaden om omsetting av mat produsert fra SYHT0H2 soya eller inneholdende ingredienser produsert fra SYHT0H2 soya.

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av SYHT0H2 de bruksområder det søkes om.

Konklusjonen er basert på
i) manglende dokumentasjon av helse og miljøeffekter med SYHT0H2 soya
ii) bruken av føre-var prinsippet ved kunnskapshull og vitenskapelig usikkerhet.

Assessment of the technical dossier submitted under EFSA/GMO/NL/2012/111 for approval of SYHT0H2 from Syngenta Crop Protection AG

Submitted to

Direktoratet for Naturforvaltning

by

Lise Nordgård, Idun Merete Grønsberg, Conny Tummler, Vinicius Vilperte
Centre for Biosafety – GenØk
March 2013

SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL/2012/111

As a designated National Competence Center for Biosafety, our mission at GenØk in advice giving is to provide independent, holistic and useful analysis of technical and scientific information/reasoning in order to assist authorities in the safety evaluation of biotechnologies proposed for use in the public sphere.

The following information is respectfully submitted for consideration in the evaluation of product safety and corresponding impact assessment of SYHT0H2, setting out the risk of adverse effects on the environment and health, including other consequences of proposed release under the pertinent Norwegian regulations.

We have targeted our critique to address the information needs under the relevant provisions that relate to our particular area of competence in biotechnology assessment as comprehensively as possible. Lack of commentary on our part towards any information under consideration should not be interpreted as specific endorsement of that information.

Specific recommendations

Based on our findings, we propose a number of specific recommendations, summarized here and detailed in the critique below.

- Considering recent scientific findings in an article published by Podevin og du Jardin, "Possible consequences of the overlap between the CaMV 35S promoter regions in plant transformation vectors used and the viral gene VI in transgenic plants", the regulator is encouraged to ask the Applicant to extend the molecular characterization of the event by examining the possibility for different RNA variants, fusion proteins and partial expression of P6.

- The regulator is encouraged to ask the Applicant to demonstrate the lack of interactive effects between transgenic proteins in this stacked event through proper scientific testing and evidence gathering, rather than justify the lack of testing based on assumptions-based reasoning of no effects.

- The regulator is encouraged to ask the Applicant to provide additional data using a comprehensive set of smaller probes in order to evaluate the genetic stability of the event; southern blot studies for generational stability should follow the same methodology as the others southern blot analysis (i.e. using the same probes); longer exposure times for Southern Blots are recommended if marker, sample or control bands are not clearly distinguishable; agarose gel pictures of the PCR fragments as well as the electropherograms should be provided

- The regulator is encouraged to ask the Applicant to include generational sequencing studies.

- The regulator is encouraged to ask the Applicant to use the plant version of the protein instead of the bacterial version in analyses to get the most authentic results.

- The regulator is encouraged to ask the Applicant to provide better figures for those where the appearance of weak bands makes it difficult to analyze the results and draw the right conclusions.

- The regulator is encouraged to ask the Applicant to include a 90 days feeding study.

Overall recommendation

From our analysis, we find that the deficiencies in the dossier do not support claims of safe use, social utility and contribution to sustainable development of SYHT0H2. **Critically, the Applicant has not included any of the required information to assess social utility and sustainability as required in Appendix 4 of the Norwegian Gene Technology Act, which would be necessary for consideration of approval in Norway.** Hence at minimum, the dossier does not comply with the informational requirements under Norwegian law. A new application or reapplication should only be reconsidered with the delivery of the information requests recommended here, including any additional information deemed significant by the Norwegian authorities.

Therefore, in our assessment of SYHT0H2, we conclude that based on the available data, supplied by the Applicant, the Applicant has not substantiated claims of environmental safety satisfactorily or provide the required information under Norwegian law to warrant approval in Norway at this time.

ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/DE/2010/86

About the event

The genetically modified soybean line SYHT0H2, developed by Syngenta Crop Protection AG, has been produced *Agrobacterium tumefaciens* – mediated transformation.

This genetically modified soybean line SYHT0H2 is modified to facilitate the control of weeds by providing tolerance to HPPD-inhibiting herbicides, such as mesotrione, due to the *AvHPPD-03* gene and to herbicides containing glufosinate ammonium due to the presence of the *pat* genes.

Assessment findings

Herbicides

The *avhppd-03* gene derived from oat (*Avena sativa L.)* encodes a p-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme that catalyzes the formation of homogentisic acid, the aromatic precursor in plastoquinone and vitamin E biosynthesis. This kind of herbicides constitutes one of the newest commercially available herbicide classes for use in different cereal crops (Beaudegnies et al. 2009, Hausman et al. 2011). A study by Hausman et al (2011) demonstrates that some weed already has evolved resistance to HPPD-inhibiting herbicides.

The *pat-03-01 and pat-03-02* genes derived from *Streptomyces viridochromogenes* confers tolerance to herbicides containing glufosinate-ammonium, a class of herbicides that are banned in Norway and in EU (except a limited use on apples) due to both acute and chronic effects on mammals including humans. Studies have shown that glufosinat ammonium is harmful by inhalation, swallowing and by skin contact and serious health risks may result from exposure over time. Effects on humans and mammals include potential damage to brain, reproduction including effects on embryos, and negative effects on biodiversity in environments where glufosinate ammonium is used (Hung 2007; Matsumura et al. 2001; Schulte-Hermann et al. 2006; Watanabe and Sano 1998). According to EFSA, the use of glufosinate ammonium will lead to exposures that exceed acceptable exposure levels during application.

Recommendation:

- The Applicant should consider that we find that it would be ethically incongruous and a double standard of safety for Norway to ban the use of this herbicide domestically as a health concern, but support its use in other countries.

Stacked events
If more than one gene from another organism has been transferred, the created GMO has stacked genes (or stacked traits), and is called a gene stacked event.

Stacked events are in general more complex and it has been an increased interest in the possible combinatorial and/or synergistic effects that may produce unintended and undesirable changes in the plant – like the potential for up- and down regulation of the plants own genes. Interactions with stacked traits cannot be excluded that the group of expressed toxins in the plant can give specific immunological effects or adjuvant effects in mammals (Halpin 2005, Schrijver et al, 2007).

Recommendation:
- The Applicant should provide direct evidence of the lack of combinatorial effects arising from the expression of the stacked proteins in the plant, instead of relying on the assessment of non-harm of the target genes existing independently, before a conclusion of safety can be scientifically justified.

2. Molecular characterization

2.2.2 Information on the sequences actually inserted/deleted or altered

A paper by Podevin and du Jardin with the title; "Possible consequences of the overlap between the CaMV 35S promoter regions in plant transformation vectors used and the viral gene VI in transgenic plants", was recently published in GM Crops and Food 3:1-5.

This paper has created a discussion related to if past approvals of GM events have overlooked key safety questions related to the use of the Cauliflower mosaic virus 35S promoter (P35S) in GM plants. In the article Podevin and du Jardin state that some P35S variants contain open reading frames that when expressed could lead to "unintended phenotypic changes. Gene VI encodes the multifunctional P6 protein that can be divided into four domains (Li and Leiser, 2002). Functions of P6 include nuclear targeting (Haas et al. 2008), viral particle binding and assembly (Himmelbach et al. 1996), si- and ds-RNA interference and interference suppression (Shivaprasad et al. 2008) and transcriptional transactivation (Koybashi et al. 2004; Palanichelvam 2001).

The 521bp P35S version inserted into SYHT0H2 soybean corresponds to bp 6914-7434 of the CaMV genome. This results in an overlap with gene VI. The applicant should therefore be required to study the presence of partial P6 protein and the possibility of chimeric proteins containing P6 fragments.

In addition to the CaMV 35S promoter SYHT0H2 soybean also contains sequences from Cestrum Yellow Leaf Curling Virus (CmYLCV), Figwort Mosaic Virus (FMV) and Tobacco Mosaic Virus (TMV). As in the case of the Cauliflower mosaic virus the Figwort Mosaic

Virus sequence indicate an overlap between its own gene VI and the promoter (Richins et al 1987). In light of the Podevin and du Jardin findings the present viral sequences should be examined carefully to exclude possible overlaps with other viral genes.

A study by Rang et al. (2005) revealed the possibility for read-through of the NOS terminator in GTS 40-3-2 soybean resulting in four different RNA variants with the potential to express unknown EPSPS fusion proteins. With respect to the fact that five NOS terminator sequences are present in SYHT0H2 soybean the possibility for read- through resulting in different RNA variants and potential fusion proteins should be studied carefully.

The 7914 bp SYHT0H2 soybean insert consist of a truncated copy and an inverted partial copy of the pSYN15954 T-DNA (Fig.A.2.1.3-3). Both of these copies contain the 35S enhancer sequence and a complete or truncated P35S. Xie et al. (2001) and Zhang et al (2008) describe that the unidirectional P35S can become bidirectional if a minimal promoter (that is essentially a TATA box region) is located at its 5' end in opposite orientation. Considering the arrangement of the P35S and the 35S enhancer in SYHT0H2 soybean there is a possibility for the P35S promoter to become bidirectional potentially resulting in diverse RNA variants.. Furthermore Zhang et al point out that bi-directional promoter using the same transcriptional factors for transcription in two directions might lead to competition for these transcriptional factors. This might influence the transcription efficiency for one of the genes and therefore protein synthesis.

Although translation of the inverted partial copy of the pSYN15954 T-DNA (Fig.A.2.1.3-3) into proteins is not expected, transcription of RNA variants (e.g. siRNAs, miRNAs) could take place. The siRNAs (21 – 24nt in length), for example, can bind to homologous RNAs due to the complementary base pairing. siRNA can incorporate into a large protein complex called RISC (for RNA induced silencing complex), which contains a ribonuclease that cleaves the target RNA to which the siRNA guide has bound, triggering degradation of the target (CERA, 2011). This process could lead to silencing of the functional gene that was inserted. Since the inverted partial sequence is complementary with the functional one, studies examining the possibility of RNA variants should be performed, using "omic" technologies (Heinemann et al, 2011).

The applicant states under point 2.2.2e that BLASTN analysis "indicated that the SYHT0H2 soybean insert does not disrupt any known endogenous soybean genes". The supplied information is however insufficient to support this claim. The tables Appendix A and C of Appendix A.2-10 show the top 10 results of the BLASTN analysis of 1000bp flanking the 5' and 3' region of the insert. For the 5' analysis the top 7 hits are not of soy origin and the top score with a soy sequence showed only a 22bp alignment (out of the 1000bp 5' flanking sequence). The 3' analysis showed a 145 bp alignment (out of the 1000bp 3' flanking sequence). Furthermore the applicant states under point 2.2.2d that "the SyHT0H2 soybean

insert had been integrated into a chromosome within the soybean genome". Detailed information on the integration site is not apparent. The applicant should use all available sequence databases and if necessary acquire more sequence data in order to supply information on "location(s) of the insert(s) in the plant cells (integrated in the chromosome, chloroplasts, mitochondria, or maintained in a nonintegrated form)" as required by Norwegian regulation.

The size of some probes used in the Southern Blot analysis is considered too long (backbone probe 5334bp, *avhppd*-03 probe 1320bp, CMP promoter and TMV enhancer probe 727bp, 2.7 and 2.9kb partial T-DNA probes 2661bp & 2909bp). That can lead to false negative results since the strength of the interaction between probe and target is based on the number of bonds that form between the single strand od DNA (probe) and the matching recombinant DNA (target). A long probe that binds perfectly to a short fragment will not bind strongly and might be washed of depending on the stringency of the wash. Especially for the analysis of backbone sequences in SYHT0H2 soybean (section 4.8 in Appendix A.2-3) a probe of over 5kb is not recommendable since small fragments might not be detected.

Appendix A.2-4 (p.22) covers the Southern Blot analysis for genetic stability studies. Only two probes (2.7 and 2.9kb partial T-DNA probes) are used for this analysis. Neither of these probes was used in the initial Southern Blots (A.2-3) and none of the probes used in the initial Southern Blot analysis were included in the genetic stability studies. Furthermore the Southern Blots were conducted with the two probes combined in the hybridization solution. By using this approach it is not certain that both probes are binding or if indeed it is only one fragment binding resulting in the Southern Blot bands. The presence of additional inverted partial copy that results in a second binding site for the 2.9kb T-DNA probe (Fig.3 Appendix A.2-4) would result in 2 bands of the expected sizes even if the 2.7kb T-DNA probe does not bind at all. The "complete coverage of all the DNA sequences" as stated under point 2.1.3. can consequently not be assumed.

Southern Blot should not be the only method used for the analysis of the genetic stability since they can only confirm the gross structure and copy number of the insert. Since small rearrangements, small deletions and point mutations that might result in the formation of new ORF or changes in the expressed protein will not be detected (De Shrijver et al 2007). The use of molecular profiling techniques (Heinemann et al, 2011) is highly recommended.

In Appendix A.2-3 (p.46), figure 10 shows a weak band on 4,8kb. This sequence has two probe binding sites, so it shouldn't be weaker than the other bands. A longer exposure time for some Southern Blots (e.g. Appendix A.2-3 Fig.19 and Appendix A.2-4 Fig.5A-C) is recommendable since some of bands in the controls or the molecular markers are very faint which makes the interpretation of the results more difficult.

For the insert sequence analyses (Appendix A_2.7), agaroses gel pictures from the PCR fragments that were sequenced are not available. The electropherograms are also not available, therefore is not possible to check the quality of the sequences.

The sequencing studies were conducted only with plants from one generation. Since Southern blot analyses for four generation were conduct, and this analysis is not able to detect small rearrangements, sequencing analysis should have been conducted as well.

Recommendation:

- Considering recent scientific findings the Applicant should extend the molecular characterization of the event by examining the possibility for different RNA variants, fusion proteins and partial expression of P6.
- The Applicant should provide additional data using a comprehensive set of smaller probes in order to evaluate the genetic stability of the event; southern blot studies for generational stability should follow the same methodology as the others southern blot analysis (i.e. using the same probes); longer exposure times for Southern Blots are recommended if marker, sample or control bands are not clearly distinguishable; agarose gel pictures of the PCR fragments as well as the electropherograms should be provided
- The use of molecular profiling techniques would allow a more thorough study of the insert genetic stability over multiple generations..

4.0 Toxicological assessment

The toxicological assessment of SYHT0H2 were based on findings related to

- Level of the proteins newly expressed
- Presence of other new constituents
- Possible changes in levels of endogenous constituents
- Impact of other changes in composition
- 28 day repeated dose oral toxicity study in rodents (OECD Guideline for the testing of chemicals, No.407, adopted 03 0ct 2008) and 28 day oral toxicity in rodents (U.S.EPA test guideline OPPTS 870.1100).

4.2 Safety evaluation of newly expressed proteins

For all protein safety evaluations, the *E.coli* produced versions of gene modified proteins (PAT and AvHPPD-03) were used. The aim must be to use plant derived version of these proteins. One should always go for the version actually expressed in the gene modifies species as many of the PTMs differ/vary between species, tissues, stage of development and according to environmental variables such as temperature and light intensity (Gomord, V. *et al.*, 2005; Küster, B. *et al.*, 2001).

Western blot analyses were used to show the identity (immunoreactivity) and mobility of the proteins using antibodies directed against the proteins. The polyclonal antibody directed against AvHPPD-03 recognizes a band between 51 and 64 kDa in both the non-modified soybean extract used as a control and in SYHT02. This band should have been investigated further using MS to check which protein the antibody cross-reacted with (Figure 2, Appendix 4-4). For the analysis of the PAT protein, the resolution of the membrane (Figure 2, Appendix 4-1) is not good. The membrane should have been exposed for a longer time to check the weaker bands present in lanes 6 and 7 (purified PAT from SYHT02 and bacterially produced PAT).

The bacterial version of AvHPPD had a slightly higher enzyme activity than the plant version (13 %), while the PAT protein showed a higher enzyme activity for the plant version of the protein (31%) compared to the bacterial version. The latter indicate that there is a difference between the plant and the bacterial version in the enzyme activity assay used on the performance level. This difference is not discussed further by the applicant.
Concentrations of AvHPPD-03 and PAT were quantifiable in all SYHT0H2 soybean tissues analyzed. The range of levels of the newly introduced AvHPPD-03 protein was measured in leaves, root, forage and seed at different growth stages (2.2.3 a,b). All plants treated with mesotrione and glufosinate ammonium had higher AvHPPD-03 concentrations than the non-treated samples, except for the seeds analyzed. For the PAT protein, the forage and seed have lower enzyme activities in the non-treated samples while seed, leaves and root have somewhat higher activities.

The glycosylation analyses are performed for both proteins. However, in Appendix 4-1(Figure 3) the picture is bad (weak signals), something that should have been better to be able to comment on the conclusion.

Recommendation:
- The Applicant should use the plant version of the protein in these analyses to get the most authentic results.
- The Applicant should provide better figures for those where the appearance of weak bands makes it difficult to analyze the results and draw the verifiable conclusions.

Information on stability of the protein under processing and storage conditions for the food and feed derived from the GM plant.

The applicant claims that fragments are not expected upon consumption on any processed fractions that has PAT or AvHPPD-03 present. There is however no data showing that they have analyzed this.

The structural integrity and enzymatic activity of PAT and AvHPPD-03 shows results that are not mentioning if these data are from the plant or bacterial version of the protein. One must assume that it is the bacterial version that is used for both proteins. Also, the references used for these analyses are old, meaning that the applicant refers to old data in this part.

Heat stability with loss of functional activity is also analyzed. The PAT protein looses its functional activity at 55°C. This temperature is used for soy processing and cooking. PAT is also degraded rapidly in simulated gastric and intestinal fluids (SGF and SIF) within 0.5 minutes. It is however the bacterial version which is tested. For the AvHPPD-03 protein, temperature effect on immunoreactivity is measured by ELISA (Appendix 3-8). At 37°C there is a loss of 24.9% of immunoreactivity, while at 65°C 96.9% of the immunoreactiviry is lost. The processing temperature (55°C) is not used for test of immunoreactivity. One must assume that some of the activity is left at this temperature. However, at 65°C, the immunoreactivity is not measurable.

The AvHPPD-03 protein could be detected in hulls, full-fat flour, and white flakes and defatted toasted meal processed from SYHT0H2 soybean seed but not in protein concentrate, isolate, milk and tofu. However, no protein was measurable after >30 min at 65°C. SGF /SIF degradation studies also showed that there were no intact fragments detected after SDS and western.

The PAT protein was also rapidly degraded in the SGF/SIF studies performed.

Recommendation:
- The Applicant should use clarify if is the plant- or bacterial version of the protein that is used the enzymatic activity studies.

Repeated dose toxicity studies using laboratory animals
The AvHPPD-03 protein was used in these studies and was given orally. It is however not said if it is the bacterial or the plant version that is used. One must assume that it is the bacterial one. It must be emphasized that the protein expressed in the plant should be used for such studies to give a more real situation and results.

Viability, clinical observations, body weights, food consumption, water consumption and ophtalmoscupy examinations were performed after these short studies, but it can be discussed if 28 days is enough to be able to see any changes in any of these parameters even if this conforms to the OECD guidelines. For the acute oral tests the bacterial version of AvHPPD-03 was used with no signs of toxicity. Here also, the plant version should have been used.

For the PAT protein, no toxicity studies were performed necessary due to the "long history of safe use".

A combination of the proteins in this soy stack has not been used in a repeated dose study even if they will be expressed and act together. The applicant considers that interactions between the two proteins are unlikely to happen but has not tested this. Neither has the whole food (SYHT02 soy) been tested for this purpose because this is not considered as necessary (the 90 day feed study).

In point 4.4 the applicant claims that there are no altered levels of food and feed constituents without referring to the data supporting this. The applicant therefore concludes that there is no need for further studies of this. The applicant should include the data so that this could be checked.

Recommendation:
- The Applicant should use the plant version of the protein, in these analyses to get the most authentic.
- The Applicant should include all data that are necessary to draw conclusions.

4.5 Assessment of whole food and feed derived from GM plants

The applicant claims that a 90 day rodent feeding study is not necessary due to the comparative assessments made demonstrating the safety of the proteins that have been introduced.

However, the soy SYHT0H2 will be resistant to HPPD and glyphosinate-ammonium herbicides, and thus treated with the corresponding herbicides at certain stages of growth. The increasing use of herbicides in the soybean production is a major challenge, resulting in the use of more herbicides and combination of herbicides (SIK-report Nr 809, Meyer and Cederberg, 2010).

With the background knowledge of gluphosinate containing herbicides that among others affect gluthamine synthase in mammals (and thus the glutamate neurotransmitter recycling), together with the fact that HPPD containing herbicides affect the tyrosine catabolism in

mammals (Shaner et al 03), the combination of these herbicides that are used on the soy stack should have been tested at authentic concentrations in a long time feeding trial to look for unforeseen effects on vital organs. This is important as gluphosiate containing herbicidies have been suspected to interfere with important biological pathways in studies using mice fed with GM soy (Malatesta et al 2008).

From the US, the use of epsps-transgenic plants has led to increased use of glyphosate compared to conventional plants (Benbrook 2003). In a recently published study by Seralini et al (Seralini et al 2012) the authors concludes that long term exposure of lower levels of complete agricultural glyphosate herbicide formulations, at concentrations well below officially set safety limits, induce severe hormone-dependent mammary, hepatic and kidney disturbances in rats.

Recommendation: Long term exposure-/feeding studies should be included in a risk assessment before a GM plant product is released on the marked for food/feed consumption.

5. Allergenicity assessment

The "weights of evidence" approach was used for the newly expressed proteins in the assessment of allergenicity following Codex 2009 (sequence aligments, serum screening, SGF/SIF digestibility).

Neither serum screening of PAT nor AvHPPD-03 or the two in combination was considered as necessary by the applicant due to the "weight" of evidence through sequence analysis of amino acids comparing it to know allergens.

The IgE binding to soybean seed proteins from conventional and SYHT0H2 soy was analyzed using human serum with no difference found between the two. This was done with the background of soybean considered as allergenic food. Comparison of SYHT0H2, conventional soybean and commercial soybean reference standards indicated that there was no difference between the gene modified soy and the others in the concentration of the known allergens.

However, the combination of the two should have been considered for such screening as this is a new combination of proteins in new context (PAT and AvHPPD-03 expressed in soy). The applicant claims that interaction between the two proteins is unlikely, without providing evidence that they have tested it. Adjuvant properties of PAT and AvHPPD-03 are not considered as a challenge due to rapid degradation in SGF/SIF and low expression in soybean seed.

Recommendation:

- The Applicant should demonstrate the lack of interactive effects between transgenic proteins through proper scientific testing.

Social utility and sustainability aspects

In addition to the EU regulatory framework for GMO assessment, an impact assessment in Norway follows the Norwegian Gene Technology Act. In accordance with the aim of the Norwegian Gene Technology Act, production and use of the GMO shall take place in an ethically and socially justifiable way, under the principle of sustainable development. This is further elaborated in section 10 of the Act (approval), where it is stated that

> *"significant emphasis shall also be placed on whether the deliberate release represent a benefit to the community and a contribution to sustainable development"*.

These issues are further detailed in the regulation on consequence assessment section 17 and its annex 4. The Applicant has not provided relevant information that allows an evaluation of the issues laid down in the aim of the Act, regarding ethical values, social justification of the GMO within a sustainable development. Given this lack of necessary information for such an evaluation, the Applicant has not demonstrated a benefit to the community and a contribution to sustainable development from the use of SYHT0H2. The Applicant should thereby provide the necessary data in order to conduct a thorough assessment on these issues, or the application should be refused.

It is also important to evaluate whether alternative options, (e.g. the parental non-GM version of SYHT0H2 may achieve the same outcomes in a safer and ethically justified way.

Further, the Norwegian Gene Technology Act, with its clauses on societal utility and sustainable development, comes into play with a view also to health and environmental effects in other countries, such as where GMOs are grown. For instance, it is difficult to extrapolate on hazards or risks taken from data generated under different ecological, biological, and genetic contexts as regional growing environments, scales of farm fields, crop management practices, genetic background, interactions between cultivated crops, and surrounding biodiversity are all likely to affect the outcomes. Hence it cannot be expected that the same effects will apply between different environments and across continents.

Recommendation:

- The applicant should submit required information on the social utility of SYHT0H2 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

Conclusion

Available information for risk assessment evaluation

This evaluation is based on the Applicant's own submitted information, along with our own expertise in related fields. The relevant scientific literature is very limited in some cases, yet we have tried to extract information from the peer-reviewed literature that may inform the scientific validity of the information under consideration. In situations where lack of

knowledge, complexity and uncertainty are high, particularly in relation to unknown adverse effects that may arise as a result of approval for release of a living modified organism into the environment or food supply, the available information may not be sufficient to warrant approval. Further information may address some of these issues, however an accurate description of uncertainties provided by the applicant would provide a more useful basis for assessing the level of risk that may come with regulatory approval of the GMO, taken on a case-by-case basis.

In all cases, product-related safety testing should have an independent and unbiased character. This goes both for the production of data for risk assessment, and for the evaluation of the data.

The lack of compelling or complete scientific information to support the claims of the Applicant documented here highlights the need for independent evaluation of the dossier as performed here, including the raw data produced by the Applicant. We therefore support better transparency and independent review of information to ensure high standards within the regulatory process. This would include any information provided by the Applicant used to justify confidentiality claims on any scientific data. We encourage the authorities to insist on this level of transparency and accessibility to all scientific data (including raw data) to ensure the scientific validity of the information presented.

Overall recommendation

Above we highlight a number of issues in relation to the questionable safe use of SYHT0H2 that do not justify a conclusion of safe use, social utility and contribution to sustainable development. Critically, the Applicant's environmental monitoring plan lacks sufficient details and descriptions to support the required monitoring activities, and has not included any of the required information to assess social utility and sustainability as required in Appendix 4 of the Norwegian Gene Technology Act, which would be necessary for consideration of approval in Norway. Taken together, these deficiencies fail to address the necessary safety regulations under Norwegian Law, and thus the application is incomplete and should not be approved. A new application or reapplication should only be reconsidered with the delivery of the information requests recommended here, including any additional information deemed significant by the Norwegian authorities.

Therefore, in our assessment of SYHT0H2 we conclude that based on the available data, the Applicant has not substantiated claims of safety satisfactorily to warrant approval in Norway at this time.

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