GenØk - Centre for Biosafety

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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,

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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.

References

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