Vår ref:2013/H109
Deres ref: 2012/16929 ART-BI-DHT

Direktoratet for naturforvaltning
Tungasletta 2
7485 Trondheim
Dato: 08.02.13

Vedlagt er inspill fra GenØk – Senter for Biosikkerhet om høringen EFSA/GMO/NL/2012/109 for oljeraps 73496 fra Pioneer Hi-Bred International, Inc.

Vennligst ta kontakt hvis du har noen spørsmål.

Med vennlig hilsen,

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Vår ref:2013/H109
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Assessment of the technical dossier submitted under EFSA/GMO/NL/2012/109 for approval of 73496 oilseed rape

Submitted to

Direktoratet for Naturforvaltning

By

Lise Nordgård, Marek Cuhra, Idun Merete Grønsberg, Arinze Okoli, Conny Tummler, Vinicius Vilperte

Centre for Biosafety – GenØk
February 2013

KONKLUSJON PÅ NORSK

Vi trekker frem mangler i dossieret som ikke gir grunnlag for en konklusjon om sikker bruk, samfunnsnytten og bidrag til bærekraftighet for oljeraps 73496.

Hovedkonklusjon og anbefalinger

Genøk – Senter for Biosikkerhet viser til brev fra Direktoratet for naturforvaltning (DN) angående høring av søknad EFSA/GMO/NL/2011/109 som omfatter oljeraps 73496 for bruksområdene import, prosessering, mat og fôr.

Rapslinjen har fått innsatt en genkonstruksjon med en optimalisert form av *gat*-genet fra jordbakterien *Bacillus licheniformis.* Genet koder for GAT4621-proteinet, et N-acetyltransferase-enzym som medfører inaktivering av herbicider med virkestoff glyfosat.

Hvor spesifikk er acetyleringen? Har søker undersøkt om enzymet også virker på andre molekyler og komponenter eller om det totale acetyleringsmønsteret er endret i den genmodifiserte linjen?

Selv om forsøkene fra søkers side samlet sett peker i retning av at det ikke er negative helseeffekter ved å benytte linje 73496 i mat og fôr, mener GenØk at slike effekter først kan gjøre seg gjeldende etter lengre tids eksponering.

I de senere tid har laboratorie forsøk vist at glyfosat kan føre til celleskader, blant annet i humane embryoceller. Undersøkelser har også vist en skadelig effekt på vassdrag og vannorganismer. I tillegg forstyrrer glyfosat næringsstoffomsetninga i jord.

Selv om det ikke er søkt om dyrking av Oljeraps 73496, er det muligheter for at importerte frø kan komme på avveie i ulike omsetningsledd og dermed representere en kilde for uønsket genspredning.

Informasjonen som er tilgjengelig fra søker er ikke tilstrekkelig for uavhengig evaluering av søknaden. Basert på manglende data og uavhengige studier tilgjengelig ønsker vi å påpeke at det er kunnskapshull relatert til risiko for helse og miljø ved oljeraps 73496

Søker gir ikke opplysninger som adresserer vurderingskriteriene bærekraft, samfunnsnytte og etiske aspekter som forutsettes anvendt i den norske genteknologiloven (Appendix 4) for godkjenning i Norge. 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 godkjenning av oljeraps 73496.

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av oljeraps 73496 i de bruksområder det søkes om.

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SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED EFSA/GMO/NL/2012/109

As a designated National Competence Center for Biosafety, our mission at GenØk in advice giving is to provide independent, holistic and relevant 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 73496 oilseed rape, setting out the risk of adverse effects on the environment and health, including other consequences of proposed release under the pertinent Norwegian regulations.

This submission is structured to address specific provisions for an impact assessment required under the Norwegian Gene Technology Act of April 1993, focusing on the requirements in Appendix 2 - Principles for environmental risk assessment pursuant to sections 13-16 of the regulations, and Appendix 4 - Evaluation of ethical considerations, sustainability and benefit to society, cf section 17 of the "Regulations relating to impact assessment pursuant to the Gene Technology Act" of December 2005, pursuant to section 11 cf section 8. The information presented here may be applicable to more than one provision in different appendices.

We have targeted our critique to 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.

All page numbers following quoted text that is not directly referenced refers to the technical dossier "EFSA/GMO/NL/2012/109", submitted by the Applicant.

Key findings

After an analysis of many of the portions of the dossier of 73496 oilseed rape submitted by the Applicant, we outline a number of inadequacies in the information submitted that do not justify the Applicant's conclusion of safety. Our input focuses on a critique of the Applicant's dossier and covers two issues:

1. Improper assumptions, reasoning, or interpretations of data that do not support a the conclusions given, or other insufficient or missing information and/or data by the Applicant related to the dossier

2. Missing or insufficient information in relation to requirements under the Norwegian Gene Technology Act

Recommendations

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

The Direktoratet for naturforvaltning is encouraged to request the following:

1. The regulators are encouraged to fill the research gaps
2. The Applicant should submit required information on the social utility of 73496 oilseed rape and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act

Overall recommendation

Based on our detailed assessment, we find that the informational, empirical and deductive deficiencies identified in the dossier do not support claims of safe use, social utility or contribution to sustainable development of 73496 oilseed rape. **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.**

Therefore, in our assessment of 73496 oilseed rape, we conclude that based on the available data, including the safety data supplied by the Applicant, the Applicant has not substantiated claims of safety in a satisfactory manner or provided the information required information under Norwegian law to warrant approval in Norway at this time.

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ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL/2012/109

About the event

According to the developer, "73496 oilseed rape has been genetically modified to provide tolerance to glyphosate by expression of the GAT4621 protein (glyphosate acetyltransferase). The GAT4621 protein, encoded by the *gat4621* gene, confers tolerance to glyphosate-containing herbicides by acetylating glyphosate and thereby rendering it non-phytotoxic (p. 41)". The Applicant claims that the availability of 73496 oilseed rape, will provide an alternative to currently available herbicide-tolerant oilseed rape lines" (p. 46).

Assessment findings

The GAT4621 protein, encoded by the gat4621 gene

The *gat4621* gene is derived from *B. licheniformis*, a gram positive saprophytic bacterium that is widespread in nature. The gene encodes a glyphosate N-acetyltransferase enzyme (GAT) (Castle et al 2004), which belongs to a family of N-acetyl transferases known as the GNAT superfamily that have a number of metabolic functions, including detoxification (Dyda et al 2000). Members of the family use acetyl-CoA as an acetyl donor to acetylate substrates and also include aminoglycoside N-acetyl transferases, which confer resistance to antibiotics such as gentamycin and kanamycin (Vetting et al. 2005).

In general, acetylation is an important mechanism through the evolution, which can change the property of a substance and it's biological, biochemical or catalytic activity. Possible unintended effects of the introduction of the GAT4621 enzyme could be the acetylation of other amino acids and compounds. In addition to glyphosate, the GAT4621 enzyme is known to acetylate five amino acids: aspartate, glutamate, threonine, serine, and glycine. In this Application the specificity of the inserted acetyl transferases should be discussed more thoroughly. The Applicant does not mention one of the most prominent functions of GCN5 related N acetyltransferases which is the acetylation of histone proteins (Dyda et al. 2000). A thorough discussion of this topic should be provided by the Applicant since the acetylation of amino acids is an unintended effect of GAT4621 and, a potential, unintended acetylation of histones is of substantial relevance concerning chromatin structure and regulation of gene transcription (Grunstein 1997; Eberharter et al. 2005).

Recommendation: The Applicant should provide a more thorough discussion about unintended effects of GAT4621.

Glyphosate tolerance

73496 oilseed rape is modified to confer tolerance to glyphosate-containing herbicides. In recent years glyphosate has received more risk-related attention due to negative effects on both aquatic and terrestrial ecosystems (Blackburn and Boutin 2003, Ono et al 2002, Solomon and Thompson 2003), and also because of constantly increasing number of glyphosate herbicide applications since the introduction of this chemicals in 1971 (Dill et al 2010, Cuhra et al 2012). Studies in animals and cell cultures indicate possible health effects in rodents, fish and humans. Glyphosate given in the feed to pregnant female rats resulted in higher embryonic mortality and aberrations in the skeleton (Dallegrave et al. 2003). Nile-tilapia (Oreochromis niloticus) fed sublethal concentration of Roundup (active ingredient:

glyphosate) resulted in a number of different histopathological changes in organs (Jiraungkoorskul et al. 2003). Experiments with sea urchins exposed to Roundup influenced early cell divisions (Marc et al 2002), effects that have relevance to potential health effects in many eukaryotic organisms, including domestic animals and humans. Exposure to Roundup affected the CDK1/CyclinB regulator which is nearly identical in sea urchins and humans.
Glyphosate has also been shown to negatively affect the differentiation of nerve cells (Axelrad et al 2003). In human placenta cells, Roundup is more toxic than the active ingredient glyphosate (Richard et al 2005). The authors concluded that additional components of Roundup increase the biological availability and accumulation in organisms.
From the US, the use of epsps-transgenic plants has led to increased use of glyphosate compared to conventional plants (Benbrook 2012). In a recently published study by Seralini et al (Seralini et al 2012) the authors conclude that long term exposure of lower levels of complete agricultural glyphosate herbicide formulations, at concentrations well below officially set safety limits, may 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.

Molecular characterization
The Applicant states that *"the data on molecular characterization did not identify features of 73496 oilseed rape with a potential to raise any safety concerns"*. However, the absence of evidence of an effect should not be used to justify a conclusion of safety.

We have some comments:

2.2.2. Information on the sequences actually inserted/deleted or altered:

- In the Table A.2.3 (p.51), the applicant shows the expected size of the fragment when digested with the *SspI* enzyme and probed with the UBQ10Promoter probe (around 2,2kb). In the Figure A.2.9. (p.55), the applicant claims that this fragment was not detected using the probe. Then in the figure A.2.17 (p.74), the applicant claims: '*A faint band is visible on the X-ray film at about 2.2 kb in lanes 8 through 17 (this band may not appear on the image or printed copy)'*. If the fragment was not detected, why does the applicant affirm that the band is present on the southern blot gel?

- The size of the majority of the probes is considered too long. 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. This might be the reason why the fragment listed above is not detected or not clearly visible in the Southern Blots. The best probe is one that approximates the size of the target sequence and does not exceed approximately 500 nucleotides in length.

- In the table A.2.2 (p.50) it is indicated that the promoter probe is "comprised of two non-overlapping labeled fragments that are combined in the hybridization solution". By using this approach you cannot be certain if both probes are binding or if indeed it is only one fragment binding resulting in the Southern Blot band (this might also be a reason why the applicant does not detect one of the expected fragments (Fig. A.2.9 – p.55) after *SspI* digestion). The complete coverage of the plasmid sequence as stated in 2.2.2a can consequently not be assumed. A combination of probes in the hybridization solution is therefore not recommendable.

- The stripping and re-hybridization of Southern Blot membranes as explained in Annex 4 is not recommendable if consecutively used probes, in this case the gat4621 and the terminator probe, detect a DNA fragment of the same size (Fig. A.2.7 (p. 53) and Fig. A.2.8 (p.54), as well as Fig. A.2.17 (p.74), Fig. A.2.18 (p.75) and Fig. A.2.19 (p.76)) since the stripping of membranes does not always result in the complete removal of the previous probe. If the stripping of the membrane was incomplete and the membrane is re-hybridized with a new probe binding to a fragment of the same size this will result in false positive results.

- In the figure A.2.19 (p.76), the applicant claims: *'A faint band is visible on the x-ray film at about 1.1 kb in lanes 8 through 17 (this band may not appear on image or printed copy)'*. After thorough examination of the indicated size range light shadows can be observed in some lanes but not clearly distinguishable bands. The note that these bands are visible on x-ray is not a sufficient documentation. If the bands are not clearly visible in the submitted documents other pictures or longer exposed x-ray films should be selected to adequately document the observed results. A statement that these bands are "visible on x-ray film" is not enough. Also,

a band of 676bp should be visible on the positive controls (lanes 1, 2, 19 and 20) according table A.2.3 (p.51). A weak binding of the probe due to its length (as described previously) might be an explanation for the weakness/ absence of the bands.

- In the Plasmid Backbone analysis (Fig. A.2.10 - p.56) shadows are visible (lane 4, 10 and 15). A longer exposure time of the X-ray film could clarify whether those shadows are spots on the membrane resulting from membrane handling etc. or if they are an actual band and therefore potential left-overs of the plasmid backbone.

- Sequencing of the 5' flanking region suggests the disruption of the tpt gene, encoding for a triose phosphate transporter, by the insertion. qRT-PCR confirmed a 7-fold transcription reduction of this specific *tpt* gene and a 50% reduction of the overall transcript levels of the *tpt* gene family in comparison to the control plants (p.63, 64). The applicant states that this "may not be biologically meaningful" and that "other copies of the *tpt* family may compensate for the loss of expression of the PG-*tpt* gene" (p.64). Only further research over multiple generations and under stress conditions can confirm these assumptions and verify that the disruption of this specific *tpt* gene has no negative implications for the plant.

Recommendation:

- The Applicant should provide additional data using a comprehensive set of smaller probes in order to evaluate the genetic stability of the event.
- Some interpretations arising from irregularities in the Sothern blot gels are equivocal. The Applicant should provide new and better pictures of the gels that make it possible to do exact interpretations.
- Stripping and re-hybridization of Southern Blot membrane is not recommended if consecutively used probes detect a DNA fragment of the same size. The Applicant should be aware that this may result in false positive results.
- Further research over multiple generations and under stress conditions should be performed to confirm and verify that the disruption of the specific *tpt* gene has no negative implications for the plant.

Complete Coverage of Sequences in Annex PHI-2009-134

- Some regions of the inserted DNA were not covered by the probes and the reasons are explained on pages 4-6.

  Regions 1 and 2 belong to the UBQ10 promoter, which has 1,3kb length and is covered by two probes. In region 1, the applicant claims that *'This region has a sequence with a relatively low GC content (32%)'* and that would affect the design of the primer. Since the sequence for the 5'flanking region was available, the primer location could have been moved upstream the 5' flanking region and region 1 would have been covered by the probe. The same applies for region 2. So, instead of excluding some regions from the analyses, the applicant could have moved the primer locations as well as split the probe into three parts, which would cover the whole fragment with adequate length (~450bp) and overlapping each other.

- This is also the case for the other regions. If the primer locations were moved either upstream or downstream the fragment, all the regions could have been covered and the probes would have an adequate length.

- On page 6 the applicant claims: '*…each of the sequences within the six regions identified above are in fact represented as smaller contiguous sequence regions contained within one or more of the probes used. For instance, although Region 1 is not included in the UBQ10 promoter probe used, there are multiple regions throughout the PHP28181 plasmid with homology to this region (6 to 10 bases matching; Table 3) and these identified regions of homology are included in the probes that were used in the Southern blot analyses'*. The sequences cannot be analyzed individually; each of the homologous regions throughout the PHP28181 is inserted in different locations and with different sequences both upstream and downstream.

Recommendation: The Applicant should include more primers/ probes to be sure that the whole fragment in order to verify the integrity of the inserted sequences.

Annex PHI-2010-086/040

- The study was conducted only with plants from the T2 generation. Since Southern blot analyses for four generation were performed, and this analysis is not able to detect small rearrangements, sequencing analysis should have been conducted as well.

- The Annex PHI-2009-217 is not available. This annex contains the plant growth conditions. It also contains the data from the 'Confirmation via Event-Specific PCR Analysis (p.9)' from the samples used on the sequencing studies.

- Primers 10-O-3386 and 10-O-3388 were used to amplify the inserted fragment, resulting in a 2452bp amplicon. No figures of this PCR are available. This is also the case for the primers 10-O-3440 and 10-O-3369 (used to amplify the 5' flanking region), and the primers 10-O3357 and 11-O-4062 (used to amplify the 3' flanking region).

- The fragments generated by the 3 pairs of primers are bigger than 2kb. Performing the sequencing reaction with *the ABI BigDye v3.1 terminator* chemistry and analyzing the results on an *ABI 3730xl capillary sequencer* (as mentioned in this annex) would give sequences with high quality only for up to 1kb. Since the fragments are bigger than 2kb, intern primers should be used to ensure the quality of the sequences. If intern primers were used, their sequences are not available.
- The applicant does not provide the electropherograms for the sequencing, which are necessary for confirming the quality of the sequences.

Recommendation: The Applicant should provide all the data necessary to do a proper independent evaluation and verification of the results obtained.

Section 2.2.3. Assessment of the newly expressed protein

- The GAT4621 protein is expressed and under control of a constitutive promoter (UBQ10 from Arabidopsis thaliana). It is expected to find the protein in all analysed tissues. Both conventional herbicide treated, near isoline conventional herbicide treated and glyphosate treated oil seed rape were analysed. Seed is the most relevant tissue for the expression analysis as it is used for food/feed. Enzyme activities were tested with ELISA using monoclonal antibodies tested for cross reactivity with 4 Cry proteins variants and 5 other proteins. Glyphosate herbicide treatment of the plants seem to have no effect on the expression of the GAT4621 protein.

Sections 4 and 5. Toxicity and allergenicity assessment

- The GAT4621 protein is a novel protein and therefore evaluated for its potential toxicity and allergenicity. Also, an unintended increase in three anminoacids (N-acetylaspartate, N-acetylglutamate and N-acetylthreonine) is found in the new oilseed rape event GAT4621. Therefore, the GAT4621 protein, the three aminoacids and whole food/diet prepared from this event is assessed.

- The GAT4621 protein used in the toxicological assessment of the newly expressed protein is produced in *E.coli*. The applicant states that the isolated plant version is inactive after purification. The applicant provides no explanation for this. From our point of view, the plant version should be used for such purposes even though the concept of equivalence is proven by structure analysis (sequencing). Plants and bacteria do differ in their post-translational processing of proteins, and this is not considered here.

- The applicant also states that the *E.coli* version of the GAT protein has the enzymatic activity and substrate specificity as expected for the plant version of the protein. However, the applicant has not been able to analyse or test this due to the problem with the inactive, purified plant version of the protein.

- The temperature range of the protein is analysed and it is most probably the microbial version of it that is tested (this is not emphasized). The range of activity appears quite narrow (36.6-42-5 °C), so the enzyme seem to be temperature sensitive. This is relevant for the processing of the oil seed rape seeds for use in food (and feed?) which includes steam treatment at around 100°C, resulting in total loss of enzyme activity and no measurable level of the protein. The protein does however seem to be stable at room temperature (section 4.2.c) making the protein available (and active) if consumed unprocessed.

- The protein is also rapidly digested in simulated gastric fluid which is used as an indication of no toxicity/allergenicity. Repeated dose toxicity studies were performed in laboratory animals (28 day study in mice). Here, the bacterial version of the protein was used. For the highest target exposure level (1000 mg/kg body weight) female mice were found to have higher body weight gains, higher mean food efficiencies and higher locomotor activity patterns than their male counterparts. This is however said to be within the historical control data range and not related to the test material. It is however interesting that only the females are affected, and this should be analysed further. In males, total bilirubin is significantly different for the highest exposure level, but this is also said to be within the normal range and not an effect of the test substance. Another issue is the low number of animals used in this study; only 5 animals per

- sex is analysed. A higher number of experimental animals would thus be recommended for proper statistical analysis.

- The applicant also states that the protein expressed in oilseed rape is the same expressed in maize (98140 Maize), but there is no data verifying this. An immunoblot or SDS gel demonstrating this claim is not provided.

- The three elevated amino acids (N-acetylaspartate elevated 500 fold, N-acetylglutamate 30 fold, and N-acetylthreonine 4 fold) were also tested for toxicity using acute toxicity studies and 28 day repeated dose studies. Other tests were also performed concluding that no adverse effect of the elevated amino acids was found. A repeated 90 day feeding study in rats was also performed with the whole feed of oilseed rape resulting in no clinical signs of toxicity for neither of the feed used.

- The allergenicity studies with rapid degradation in simulated gastric fluid; low level of expression, lack of glycosylation and low thermal stability is used as history of safe use and evidence of lack of allergenicity. However, these results are based on the microbial version of the protein that is tested here and not the plant derived version. Also, as oilseed rape is not considered to be an allergenic plant, the allergen repertoire of the 73946 oilseed rape is not tested further! There is however indications that oilseed rape can induce allergenicity (McSharry, 1992; Fell et al 1992, Hemmer et al 1997) without being able to connect this to a particular part of the crop (Parrat et al 1995). The potential allergenicity of the plant after introduction of new proteins expressed in a new context should therefore be analysed further.

Recommendation: The Applicant should perform analysis of potential allergenicity of the plant-derived proteins in 73946 oilseed rape.

Data on the possible relationship of the Gene products with known Toxins, Anti-nutrients and Allergens (page 42); and up-to-date bioinformatics search for homology (page 146)

The conclusions by the applicant on page 42 vis *"From these analyses it is concluded that GAT4621 shows no relationship to known toxins, antinutrients or allergens"* ; and on page 146 vis *" To summarize, there were no significant alignment returned between the GAT4621 protein and any protein exerting a normal metabolic or structural function"* are not substantiated by the level of analyses conducted. The following concerns should be addressed by the applicant:

- A motif/domain search such as FARRP12 evaluation using 8 contiguous identical amino acid stretches was conducted only for allergen identification but not for the identification also of toxins and antinutrients because searches against specific toxins and antinutrients databases were not conducted. A sequence homology search alone for toxins and antinutrients is grossly inadequate and cannot substantiate the claim of *"…shows no relationship to known toxins, antinutrients…"* In the very least, both sequence and domain searches using both local and global alignment algorithms should be used to search specialized databases generated using relevant strings such as 'toxin', 'toxic', 'toxigenic', etc, (Podevin and du Jardin, 2012).

- Percentage identity as used by the applicant is too restrictive and has the possible effect that it could lead to false negative, a more robust parameter, similarity score, should have been used,

which would include not just identical sequences but also sequences that differ in just few amino acid residues but which would not affect the eventual protein structure and function. In this way, chances of identifying matches to potential allergens, toxins and antinutrients would be increased. It is noted that the applicant selectively reports identity scores, similarity scores and cut-off expectation scores for bioinformatics analyses of sequences; similarly a selective use of local and global alignment algorithms (BLASTp & FASTA35 respectively) [Section A2.1.2c, page 42; and Section 4.2a(b), page 146]. An explanation for the need for the selective applications of these paramaters is required as they have important influences on the deductions from the analyses of sequences.

- Functional domain analyses should also be conducted by the applicant in the assessment for GAT4621 and proteins exerting normal metabolic or structural functions (Section 4.2a(b), page 146].

  It is worth mentioning that if a domain search shows no hit against known toxin, allergen or antinutrient, it should not be concluded that potential matches to toxins/allergens/antinutrients may not be found in the future because the domain databases domains are constantly updated.

Recommendation:

- The Applicant should include searches against curated domain databases for potential toxins and antinutrients.
- The Applicant should generate and use specialized databases for toxins and antinutrients in the evaluation of potential toxins and antinutrients.
- The Applicant should include similarity and expectation scores should alongside identity scores in all cases.
- The Applicant should give explanations for selective use of local and global alignment algorithms.
- Conclusions based on sequence analyses should give a clause with an explanation that evaluations for allergens, toxins, antinutrients would be a continual process as databases become updated.

Comments on application part D: Post market monitoring

We have reviewed the scientific basis presented by the applicant supporting the application on specific questions concerning "post market monitoring" and environmental risk-assessment. We see that there are important issues to highlight.

- The Applicant partially confirms that there is a substantial risk of biological contamination from 73496 oilseed rape escaping into European environment and establishing viable populations. The applicant concludes that this is highly probable (application part D, p: 193-201), but in the opinion of the applicant the GT-variety will not show increased persistence or occurrence as agriculture pest (weed), *unless* co-technology herbicide glyphosate is applied. It is established that glyphosate is the major herbicide on a global scale with estimated 0.5-1.1 mio tonnes of active ingredient applied annually (Pollak, 2011; Scekacs et al., 2012). Thus we conclude high probability of relevant biochemical selective pressure supporting arguments of precautionary approach.

- The risk factors associated with the EU import of 73496 oilseed rape can be categorized as relating to human health, feed use, environment and agriculture. The application does not apply for cultivation and deliberate release of viable seed, but never the less some questions relate to the importation, storage and within EU transportation of such viable seed. These questions should be addressed in a environmental monitoring plan, which is a formal requirement in this application. Given the quantities of transgenic material to be imported, it is important to establish routines and systematic approaches within the logistics of storage and transportation, to avoid spillage and contamination. Typically such material is bulk-carried, with semi-open systems for handling and distribution.

- Several questions remain unresolved, one of them survivability and dissemination of 73496 oilseed rape seed from accidental spills. Biotech industry has acknowledged *"Oilseed rape dissemination can occur by means of seeds and pollen. The seeds have no special or specific adaptations to facilitate widespread dispersal (they are not wind transported and have no structures to allow them to stick to animal fur) and so any shattered seed will remain in close proximity to the site of production. Further dissemination may occur by means of fauna or machinery."(Monsanto Europe, 2012, p. 27, section 4a).*

- Additionally, well-established scientific knowledge of seed dispersal mediated by birds and other organisms (Howe and Smallwood, 1982) highlight the need for better focus on this aspect.

- Continued traditional agriculture coexistence and implications for such coexistence from transgenic volunteers of *Brassica* such as 73496 oilseed rape and avifauna dispersal of viable commercial seed from genetically modified crops has recently been studied in transgenic crops in genera (Cummings et al., 2008) and in canola *Brassica napus* specifically (Twigg et al., 2008). Potential dispersal of viable seed by such vectors should not be underestimated and has to be adressed by the applicant.

- Although some studies estimate the dispersal range of viable *B. napus* seed as generally less than 10 kilometres in the species of birds investigated (Wood duck, *Chenonetta jubata*) (Twigg et al., 2008), other observations indicate that the potential dispersal range could be much larger in other bird species. Recent evidence also indicates that transgenic glyphosate tolerant *B. napus* originating from Canada, is dispersed into the USA by migrating geese. The

issue has only been realized recently and mainly since the geese-mediated volunteers of *B. napus* turn up as resistant weed in agriculture of other glyphosate tolerant transgenic plants, in this specific case transgenic GT sugar beet in North Dakota.

- There are aspects of other potential interactions of 73496 oilseed rape with organisms in the ecosystem where it is usually grown or used elsewhere, including toxic effects on humans, animals and other organisms. The applicant does not give information on possible effects of a main issue concerning GT crops, namely the potential effects on health and environment from the substantial associated use of glyphosate, this herbicide being an unavoidable and integrated element of the cultivation of these varieties. Such potential effects of glyphosate use are not only restricted to the environment and ecosystem where the glyphosate-tolerant varieties are be grown, but also has the potential to affect nutrient composition of the crops (Zobiole et al., 2010, 2011) as well as inducing substantial levels of pesticide residues and metabolites of pesticides in seed (Duke et al., 2003) thus influencing the quality of the produce.

- The applicant does not provide the environmental monitoring plan requested for importation into the EU-area of viable seed of transgenic plants. The environmental monitoring plan should also take into consideration potential environmental consequences of spillage during import, transportation, storage, handling and processing of 73496 oilseed rape within the area of the European Union. Such avoidance on the part of the applicant should not be accepted by the regulator, especially as the Commission Decision (EU 2005) does not specify the extent and details of the monitoring plan.

- The environmental monitoring plan has high relevance and must ensure future coexistence of local European varieties of *B. napus* and related species potentially subject to contamination from the transgenic varieties. Even if the local varieties currently under cultivation may not reflect the original diversity of *Brassica* in the European centre of origin, the varieties are an important traditional part of agriculture in Central and Northern Europe.

- Recent evidence (Scaefer et al., 2011) documents wide-spread establishment of feral populations of transgenic canola in the US. The researchers found 347 plants out of a total of 406 volunteers collected, to be carrying either CP4 EPSPS or PAT (confers tolerance to glufosinate) gene. Interestingly, even two instances of plants having both transgenes were found, despite the fact that such canola varieties with stacked traits had not been released commercially at the time. The researchers comment that; *"These observations indicate feral populations are reproducing and have become established outside of cultivation making this the first report in the U.S. of established populations of genetically modified organisms in the "wild". As such, these observations have important implications for the ecology and management of native and weedy species, and as well as for the management of biotech products in the U.S."* (Schaefer et al., 2010). There is no doubt that these are very important findings, not least since such discoveries of feral populations of canola bearing biotech traits now have been reported from Canada, Great Britain, France, Australia and Japan (Schaefer et al., 2011). We also conclude that this evidence has not been weighted sufficiently by EFSA in recent decisions on similar transgenic cultivars (EFSA 2012).

- Analysis of feral populations of *Brassica* in the EU (Reuter et al., 2011) and elsewhere (Schaefer et al., 2011) has partially correlated these occurrences of transgenic varieties with

geographically defined spatial infrastructure for transport logistics such as roads and railway lines, but more surprisingly occurrence of hybrids of transgenic varieties (crosses of distinct transgenic lines) have also been confirmed (Scaefer et al., 2011). It is known that these specific hybrids are not produced commercially as stacked traits for cultivation and it has been concluded that the probable explanation for the occurrence, is wild hybridization amongst transgenic volunteers.

Recommendation: The Applicant should present a plan for monitoring of unintended release and occurrence within the potentially affected areas in accordance to the EFSA requirement

Missing or insufficient information in relation to requirements under the Norwegian Gene Technology Act

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 73496 oilseed rape. 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 73496 oilseed rape has achieved 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 73496 oilseed rape 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 support the scientific validity of the information under consideration. In situations where there is a lack of knowledge, or 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.

In all cases, product-related safety testing should have an independent and unbiased character. This applies equally for both for the production of data for risk assessment, and for the evaluation of the data. The lack of compelling or complete scientific information from the Applicant that we have 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 any policies that would require greater 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 require 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 conceptual, empirical and informational deficiencies in the dossier that do not justify a conclusion of safe use, social utility and contribution to sustainable development of 73496 oilseed rape. 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. 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 73496 oilseed rape we conclude that based on the available data, including the safety data supplied, the Applicant has not substantiated claims of safety satisfactorily to warrant approval in Norway at this time.

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