

Assessment of the scientific information submitted under EFSA/GMO/BE/2011/101 for approval of transgenic oilseed rape MON88302 from Monsanto Company.

Submitted to

Direktoratet for Naturforvaltning

by

Centre for Biosafety – GenØk June 2012



KONKLUSJON PÅ NORSK

Vi trekker her frem flere begrepsmessige, empiriske og informasjonsmessige mangler i dossieret som dermed ikke gir grunnlag for en konklusjon om sikker bruk av MON88302, om samfunnsnytte av denne nye plantevariant eller om dennes bidrag til bærekraftighet.

Hovedkonklusjon og anbefalinger

Genøk –Senter for Biosikkerhet viser til brev fra Direktoratet for naturforvaltning (DN) angående høring vedrørende rapsplanten MON88302 for bruksområdene import, prosessering, mat og fôr. Planten er genmodifisert for å kunne tåle høyere doser av sprøytemiddelet glyfosat, samt for å kunne tåle sprøyting opp mot blomstringsstadiet.

Søker gir ikke opplysninger som adresserer vurderingskriteriene bærekraft, samfunnssnytte og etiske aspekter som forutsettes anvendt i den norske genteknologiloven. I denne sammenheng er det viktig å få dokumentert om den omsøkte planten fører til mindre bruk av sprøytemiddelet, samt erfaringer med hensyn på effekter på miljø, helse og samfunnsaspekter hos bønder som dyrker den. Denne type dokumentasjon er ikke vedlagt søknad om omsetting av rapsplanten MON88302

Søker har ikke utført analyser av viktige kjemiske prosesser som erfaringsmessig vites å være aktuelle problemstillinger for denne type genmodifiserte planter (herbisidtolerans medfører akkumulering av pågjeldende stoffer).

Søker har ikke utført verken foringsstudier med forsøksdyr, eller allergenisitetstesting av plantematerialet.

Basert på manglende studier, manglende monitoreringsplaner og manglende datagrunnlag må vi påpeke at det er vesentlige kunnskapshull relatert til risiko for helse og miljø ved den omsøkte bruken av rapsplanten MON88302. Disse kunnskapshullene er spesielt relatert til usikkerhet ved:

- Overordnet risikovurdering av plantematerialet
- Komposisjonsmessig kvalitet av plantematerialet med fokus på pestisidinnhold
- Manglende plan for å unngå spredning til naturmiljøet
- Manglende plan for monitorering (miljøovervåking)

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av GM rapsen i de bruksområder det søkes om. Konklusjonen er basert på I) manglende dokumentasjon, II) faktuelle feil i søknaden, III) manglende utredning av forhold vedrørende samfunnsnytte og bærekraft, samt IV) nødvendigheten av å bruke føre-var prinsippet ved kunnskapshull og vitenskapelig usikkerhet.

Søker har ikke inkludert noe av den informasjonen omkring samfunnsnytten og bærekraftighet til MON88302 som kreves i den norske genteknologiloven (Appendix 4) for godkjenning i Norge.



SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL/2011/101

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 event MON88302, 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, of 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 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.

This submission was built in large part using the **Biosafety Assessment Tool** (https://bat.genok.org/bat/) produced by the University of Canterbury and Gen \emptyset k – Centre for Biosafety. This is a free-to-the-public resource for hazard identification and risk assessment of genetically modified organisms.

All page numbers following quoted text that is not directly referenced refers to the scientific information "APPLICATION FOR AUTHORIZATION TO PLACE ON THE MARKED MON88302 OILSEED RAPE IN THE EUROPEAN UNION, ACCORDING TO REGULATION (EC) No 1829/2003 ON GENETICALLY MODIFIED FOOD AND FEED", submitted by the Applicant.



Key findings

The applicant states that "MON 88302 provides tolerance to glyphosate during the sensitive reproductive stages of growth, and enables the application of glyphosate at higher rates up to first flower with no detectable impact to male fertility."

Furthermore the applicant states that; "The higher glyphosate rates and extended timing for applications possible with MON 88302 will enable better control of difficult to manage weeds."

The applicant also states that; "The scope of the current application is for all uses of MON 88302 as any other oilseed rape. The application also includes import and processing of MON 88302. Therefore, a complete safety assessment for MON 88302 is presented, according to Regulation (EC) No 1829/2003."

After a analysis of many of the portions of the dossier on MON88302 submitted by the Applicant, we outline a number of inadequacies in the information submitted in the dossier that do not justify the Applicant's conclusion of safety. Our input focuses on a critique of the Applicant's dossier and covers two broad 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

Lastly, Codex Alimentarius guidelines allow Norway to ask for specific data of the type we identify and recommend obtaining. Norway therefore may request such information without concern of a challenge from the World Trade Organization.



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 applicant should provide more data to verify that probes used would also detect smaller or rearranged transgenic fragments that may be integrated into host genome.
- **2.** The applicant should provide additional data using a comprehensive set of smaller probes to establish the presence or absence of backbone vector DNA sequences.
- **3.** Given the deletions reported after integration of the transgenic DNA into host genome, the applicant should provide a survey of the actual RNAs produces or absent at the integration junctions and in the DNA surrounding the insert, preferably using high throughput transcriptome sequencing techniques.
- **4.** The applicant should provide experimental evidence that any detected rearrangements, deletions or insertions do not lead to any adverse effects.
- 5. The applicant should provide more detailed methods in the expression studies.
- 6. The expression levels of CP4 EPSPS show high variation between plants. Whether this might have an effect on the glyphosate tolerance or the forage quality is not predictable but should be examined.
- 7. The applicant should use the plant produced CP4 EPSPS and not the *E.coli* produced CP4 EPSPS to assess the safety of the protein.
- 8. The applicant should present data for pesticide residues, specifically for glyphosate and AMPA
- **9.** The applicant should present results of animal feeding studies using the MON 88302 variety, instead of referring to soybean studies they conducted in 1996.
- **10.** The applicant should present results from testing for allergenicity of the MON 88302 variety instead of referring to non-representative previous studies of purified EPSPS protein.
- **11.** The applicant should elaborate on the risk of dispersal of viable seeds from spillage and via animal vectors.
- 12. The applicant should present an adequate Environmental Monitoring Plan.



- **13.** The applicant should document how this new variety will benefit society and contribute to sustainable development as required in the Norwegian Gene Technology Act.
- **14.** The intention stated by the applicant, to distribute viable MON 88302 seeds as ingredients in commercial bird-feed must under no circumstance be allowed, as this will most certainly have strong negative environmental consequence.

Overall recommendation

Based on our assessment, we find that the informational, empirical and deductive deficiencies identified in the scientific information do not support claims of safe use, social utility and contribution to sustainable development of MON88302. 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 scientific information is deficient in information required 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 MON88302, we conclude that based on the available data, including the safety data supplied by the Applicant, the Applicant has not substantiated claims of 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/NL/2011/92

About the event

The organism used for genetic transformation to produce MON88302 was Agrobacterium tumefaciens strain ABI. MON88302 produces the 5-enolpyruvylshikimate-3-phosate synthase (CP4 EPSPS) protein which confers resistance to the herbicide glyphosate, the active ingredient in the family of Roundup agricultural herbicides. In addition MON88302 utilizes a FMV/Tsf1 chimeric promoter sequence to drive CP4 EPSPS expression in different plant tissues including pollen.

Assessment findings

Herbicides

The genetically modified oilseed rape MON88302 has been modified to tolerate **higher** doses of the herbicide glyphosate than other varieties of GT-oilseed rape (such as the GT-73 variety). The applicant also states that MON88302 tolerates glyphosate applications up to later stages of growth, enabling greater flexibility for farmers who can apply herbicides up to the flowering stage.

Glyphosate tolerance

The MON88302 contains the *CP4EPSPS* gene from *Agrobacterium tumefaciens strain ABI* that confers tolerance to herbicides containing glyphosate.

Glyphosate has been heralded as an ideal herbicide with low toxicity for operators, consumers and the environment surrounding agriculture fields (Duke & Powles 2008, Giesy et al 2000), but has received more risk-related attention due to its negative effects on both aquatic and terrestrial ecosystems (Blackburn and Boutin 2003, Ono et al 2002, Solomon and Thompson 2003) and studies in animals and cell cultures indicate possible health effects in rodents, fish and humans (Marc et al 2002, Axelrad et al 2003, Dallegrave et al 2003, Jiraungkoorskul et al 2003, Richard et al 2005, Benachour et al 2007, Gasnier et al 2009)

Glyphosate kills plants by inhibiting the enzyme 5-enolpyruvoyl-shikimate-3-phosphate synthase (EPSPS), necessary for production of important amino acids. Some microorganisms have a version of EPSPS that is resistant to glyphosate inhibition. The transgene, cp4 EPSPS, used in genetically modified crops was isolated from an Agrobacterium strain. The whole idea is the combined use of the GM plant and the herbicide. Recent studies indicate that agriculture of GM plants is associated with greater overall usage of pesticides than the conventional agriculture (Benbrook 2009). Large proportions of GM agriculture is glyphosate tolerant crops (GT-cultivars) (James 2010).



A restricted number of recent publications indicate unwanted effects of glyphosate on health (Dallegrave et al 2003, Malatesa M et al 2002), aquatic (Solomon K & Thompson D 2003) and terrestric (Ono MA et al 2002, Blackburn LG & Boutin CE 2003); organisms and ecosystems. Some of these may be considered "early warnings" of potential health and environmental risks, and they should be rapidly followed up to confirm and extend the findings.

Studies in animals and cell cultures point directly to health effects in humans as well as rodents and fish. Female rats fed glyphosate during pregnancy demonstrated increased foetal mortality and malformations of the skeleton (Dallegrave E et al 2003). Mice fed GE soybean demonstrated significant morphological changes in their liver cells (Malatesta M et al 2002). The data suggested that EPSPS-transgenic soybean intake was influencing liver cell nuclear features in both young and adult mice, but the mechanisms responsible for the alterations could not be identified by the experimental design of these studies. Treatment with glyphosate (Roundup) is an integrated part of the EPSPS-transgenic crop application. Nile Tilapia Oreochromis niloticus) fed sublethal concentrations of Roundup exhibited a number of histopathological changes in various organs (Jiraungkoorskul W et al 2003). A study of Roundup effects on the first cell divisions of sea urchins (Marc J et al 2002) is of particular interest to human health. The experiments demonstrated cell division dysfunctions at the level of CDK1/Cyclin B activation. Considering the universality among species of the CDK1/Cyclin B cell regulator, these results question the safety of glyphosate and Roundup on human health. In another study (Axelrad JC et al 2003) it was demonstrated a negative effect of glyphosate, as well as a number of other organophosphate pesticides, on nerve-cell differentiation. Surprisingly, in human placental cells, Roundup is always more toxic than its active ingredient. The effects of glyphosate and Roundup were tested at lower non-toxic concentrations on aromatase, the enzyme responsible for estrogen synthesis (Richard S et al, 2005). The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation. The authors conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. They suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.

Molecular characterization

PCR and Southern Hybridization

The Applicant states: "The molecular characterization of MON88302 does not raise any safety concern and does not show any evidence of unintended changes in MON 88302." Characterization of the DNA insert in MON88302 was conducted by Southern blot, PCR and DNA sequence analyses.

Insert and copy number

To test for the numbers of copies and insertion sites of the T-DNA sequences in the oilseed rape genome, restriction enzymes and Southern blot hybridization was used. The Applicant have used probes for southern blot hybridization ranging in size from 1,3-2,3 kb (listed in figure 2 p 26). The use of long probes to detect recombinant DNA can lead to false negative results. The strength of the interaction between probe and target is based on the number of



bonds that form between the single strand of DNA that is the probe and the matching recombinant DNA that is the target. A long probe that binds perfectly to a short insertion will not be strongly bound and may be washed off depending on the stringency of the wash. The best probe is one that approximates the size of the target sequence and does not exceed approximately 500 nucleotides in length. Probes that are > 500bp means that point mutations, small deletions and rearrangements that might occur during breeding will possible not be detected (Fagard&Vauvheret 2000, de Schrijver et al 2006). This means that in this case, the applicant failed to account for potential inserts that are only partial, either smaller than the probes or with rearrangements, both of which could prevent binding of the probe and therefor detection of rDNA integrated elsewhere in the genome (Kononov et al 1997).

In general, the southern membranes provided in the dossier and also in some of its reports lack labeled markers. How can you know that the band has the expected size without using a marker?

Recommendation:

- The applicant should use a set of smaller probes (<u>www.bat.genok.org/bat</u>) and used a labeled marker in the upset.
- In general, the presented figures are acceptable. However, the southern blot figures lack a visible molecular weight marker on the membrane which should always be present.

Detection of absence of backbone vector DNA/unintended transgenes

Examination of the insert, the flanking genomic and genomic DNA insertion site was characterized by PCR and DNA sequencing. A 9 base pair insertion adjacent to the 3'end of the MON88302 insert, a 29 base pair deletion from the conventional genomic DNA occurred during the insertion of the T-DNA into the conventional oilseed rape to form MON88302, and a single nucleotide difference between the conventional counterpart sequence and the known DNA sequence flanking the 3'end of the MON88302 insert was reported by the Applicant.

The Applicant states that these molecular rearrangements presumably resulted from doublestranded break repair mechanisms in the plant during the agrobacterium-mediated transformation process (Salomon and Puchta 1998), however they do not mention any possible consequences because of these rearrangements.

Recommendation:

• Given the deletions reported after integration of the transgenic DNA into the host genome, the Applicant should provide a survey of the actual RNAs produced or absent at the integration junctions and in the DNA surrounding the insert, preferably using high throughput transcriptome sequencing techniques (Heinemann et al 2011).



ELISA

The applicant states: "The CP4 EPSPS protein expression levels ($\mu g/g dw$) determined from treated tissues of MON 88302 were comparable to those determined from untreated MON 88302 tissues, showing that glyphosate application in MON 88302 does not alter nor have any negative effects on the expression of the CP4 EPSPS protein in the plant." Information on the expression of the inserted modified sequence was conducted by ELISA.

While the mean CP4 EPSPS expression levels in untreated and glyphosate treated Mon 88302 are comparable in forage and grain there is a wide range of expression levels $120-210 \mu g/g$ dwt and $90-290 \mu g/g$ dwt for sprayed and unsprayed forage and $22-46 \mu g/g$ dwt and $22-42 \mu g/g$ dwt for spayed and unsprayed grain. Sprayed over- season leaves even had an expression range between 110 and 500 $\mu g/g$ dwt. The expression levels of CP4 EPSPS show high variation between plants. Whether this might have an effect on the glyphosate tolerance or the forage quality is not predictable but should be examined.

Further information about the time difference between the last glyphosate treatment and the sampling of the plant material is important to interpret the CP4 EPSPS expression levels.

The description of the ELISA method in the given references (Clark 2012a; Clark and Niemeyer 2010a) is not detailed enough. Important information necessary to replicate the measurements is missing like a detailed description of the protein extraction method, antibody dilutions and how many parallels of each sample that were measured. Additionally the raw data (OD-values) and standard deviations for sample parallels are not available. The inter-assay negative and positive controls are not specified.

Under point 4.6 and 5.1 as well as point 2 below table 1 of the Clark and Niemeyer 2010a report it is mentioned that samples which showed unexpected negative results or "unexpected results" during ELISA or PCR were omitted. It is not further explained what is considered an "unexpected result" or why these were excluded.

As a protein standard *E.coli* produced CP4 EPSPS protein was used. Since there might be differences in the affinity of the used antibodies for *E.coli* and Mon 88302 derived CP4 EPSPS the Mon 88302 derived CP4 EPSPS should be used for a standard curve. Figure 20. Molecular weight and purity analysis of the MON 88302-produced CP4 EPSPS shows that it was possible to purify quite high amounts of the protein and therefore it should be used as a standard in the ELISA.

Furthermore it is not specified if the used antibodies were raised against CP4 EPSPS protein derived from an *E.coli* expression system or against the Mon 88302 CP4 EPSPS. Antibodies raised against the *E.coli* derived CP4 EPSPS might in fact not be able to detect all isoforms of the Mon 88302 CP4 EPSPS possibly produced in-planta.



Recommendation:

- The methods used in the expressions studies are not detailed enough in order to make an appropriate evaluation. The expression levels of CP4 EPSPS show high variation between plants. Whether this might have an effect on the glyphosate tolerance or the forage quality is not predictable but should be examined. In addition it is not specified if the used antibodies were raised against CP4 EPSPS protein derived from E. coli or against the MON88302 CP4 EPSPS.
- In general, there is no scientific literature available on the genetic construct, the genetic stability, transgene expression products or immune-toxicological effects, in order to make an appropriate scientific evaluation.

Health effects

Regarding potential health effects the applicant claims safe use since oilseed rape in general has a long history of safe use. The applicant also claims that the event is not considered to have toxic effects to humans, animals and other organisms.

However, the data provided in the dossier do not give enough evidence that the use of MON88302 is safe from a toxicological nor allergenic point of view. No scientific studies on the plant variety in question are available in order to make an appropriate scientific evaluation.

No feeding studies have been performed in animals. No studies of allergenicity have been performed in neither animals nor humans. The applicant states that such testing is "not necessary", since the non-toxicological and non-allegenic properties of the CP4 EPSPS protein are well established (sections A 4.2.5, A 4.5 and A 5.4). Thus the applicant ignores a main point of the EFSA-risk assessment guidelines for GM-plants, namely that the risk of unwanted or adverse effects due to changes in the recipient genome must be anticipated and tested. Thus, it is not only a question of effects directly produced by the inserted CP4 EPSPS gene, but also important to evaluate indirect effects stemming from the modification itself. Here EFSA is very clear and recommends animal feeding trials to address this specific risk.

Toxicological assessment

The assessment of potential toxicity of the expressed CP4 EPSPS protein is included in the dossier with the same arguments as previous GM plants with the same inserted gene. These arguments are the ones that are most commonly used:

- History of safe use
- No structural similarity to known toxins
- No acute toxicity effects to mammals
- Rapid digestion in digestive fluid

The argument of long history of safe use is based on the "fact" that this bacterially derived protein has posed no risk to human health since its introduction to food and feeds in 1996 (Delaney et al 2008) and that the actual concentration of the CP4 EPSPS protein is very low



in food and feed. The applicant has not analysed the question of safety further because of the "weight of evidence" provided in the long history of safe use. Still, the protein is expressed in a new context in this event (expression throughout the plants development) to be able to spray it with glyphosate herbicides more frequent and at an earlier stage and should be analysed more thoroughly as it differs from the previous events where this protein is expressed.

Recommendation:

• The protein is expressed in a new context in this event and should be analysed more thoroughly as it differs from the previous events where this protein is expressed.

Assessment of the newly expressed protein

For the assessment studies, the applicant uses *E.coli* produced CP4 EPSPS as "the levels of introduced protein *in planta* usually are too low". However, they use the plant version of the protein in the molecular weight and protein purity assays, thus they are able to isolate the protein. The bacterial version is used for *in vitro* digestibility, acute oral toxicity and heat stability analysis of the protein.

The applicant provides evidence for equivalence between plant and bacterial version of CP4 EPSPS. However, the applicant should search to use the plant version of the protein in these analyses to get the most authentic results as the two proteins are expressed in bacteria and plant. This means that the protein that actually is expressed in the gene modified species, and derived from it, should be used due to the potential differences that can arise because of post translational differences between species, tissues and stages of development (Gomord et al 2005, Küster et al 2001).

The plant and the bacterially derived proteins seem to be of same size and immune-reactivity based on the results presented by the applicant. The acceptation criteria for immune-reactivity are set to +/- 35 %. These are met (24.1 % average difference is presented. The difference varies between 14.9 and 30.8 percent, depending on the concentration of the protein loaded in the gel). Higher concentrations of protein in gel gives more saturated bands and thus they are measured more equal in concentration. It therefore seems important to have suboptimal ag/protein levels in the gel to get real comparative data (bands that are not saturated).

Figure 21, showing the western blot analysis of plant and bacterially derived CP4 EPSPS protein should have been exposed longer to check the presence of additional bands caused by potential post translational activities and proteins of different size. One would also assume that the antibody used in the immunogenic reactions are raised using the bacterially derived version of the protein, raising another issue on equality and reactivity of proteins expressed from as different sources as plants and bacteria.

There should also have been a presentation of the result after protein isolation from representative food and feed containing MON88302, to verify presence/absence and immune-reactivity.

The bacterial and the plant derived CP4 EPSPS proteins were analysed for the presence of glycosylation as many eukaryotic proteins are post-translationally modified with carbohydrates (Rademacher et al 1988) while prokaryotic glycosylation is less common. The



presence of glycosylation has also been used as one of the criteria for a potentially allergenic protein as allergenic proteins often are found to be glycosylated. No glycosylation was detected of the plant version of CP4 EPSPS with the method used. The positive control is visible after 2 min exposure (Figure 5, Bhakta et al 2010). The bands presented in this figure are however quite faint. An additional figure should have been present, indicating if there are any changes to the detected signals when the membrane is exposed further/longer. The applicant uses CP4 EPSPS from bacteria as the negative control. A second negative protein should also have been added, that is not EPSPS.

The plant derived protein also shows no homology to known toxins or biologically active proteins using bioinformatics tools.

Protein stability during processing and storage (food/feed from the gene modified plant) was analysed by performing heat treatment of purified *E.coli* derived protein. The results indicate that the protein loses its activity at high temperatures, also at the relevant temperature of 75° C and higher (processing: conditioning of oil seed rape starts at 75° C). Thus they conclude that the rape seed oil does not contain the protein in question and at least not active (Dossier, p.120). But they have not actually tested the *in planta* version at the relevant temperatures, or the potential food/feed itself. What they do show is that the *E.coli* derived CP4 EPSPS protein maintains its size after the 95°C for 30 min treatment and that it loses its activity at 75° C.

The applicant has also tested the CP4 EPSPS proteins for resistance to protelytic cleavage and intactness when subjected to different pHs. The protein is demonstrated to be intact at neutral and acidic pHs. Also, the protein is rapidly degraded in simulated gastric fluids, yet another indication of low potential as an allergen or toxin. However, they have only tested the bacterial version and not the one found in plants.

In general, the presented figures are acceptable. However, some of them lack a visible molecular weight marker on the membrane (Figures 21, 22, 26).

Recommendation:

- The Applicant should use the plant produced CP4 EPSPS and not the *E.coli* produced CP4 EPSPS to assess the safety of the protein
- The figures presenting western blots of the protein should have visible molecular weight markers, and not only arrows indicating sizes.
- Activity of CP4 EPSPS protein isolated from representative food and feed should be analysed

Allergenicity assessment

Allergenicity of the CP4 EPSPS protein is tested through Codex Alimentarius, 2009 (Codex, 2009). The assessment is heavily based on that the protein is from a non-allergenic source, has no structural similarities to known allergens, is rapidly digested and not stable at heat treatment. The conclusion from the applicant is that the protein is not allergenic.

The Applicant does not discuss potential allergenicity of the plant derived version of the protein, but rely on data obtained from the bacterial version of it. Also, the statement that the



protein is not stable is not true: the protein is stable up to 95° C and for the tested 30 min (Figure 24). Low percentage of the CP4 EPSPS protein as compared to total protein is presented as one of the points in the allergenicity assessment. However, this is not relevant when it comes to allergenicity as only traces of allergenic protein in food have been found to give allergenic reactions. Interestingly, they are able to isolate protein from rape seed oil in this part of the dossier, although at low levels. This protein is however not analysed further. Only the *E.coli* version of the CP4 EPSPS protein is used for the allergenicity assessment. The Applicant also states that oilseed rape not is considered to be an allergenic plant. Very few in the population are allergic to oilseed rape plant and pollen. However, oil seed rape with CP4EPSPS is "new" in this context and should be assessed as such.

Analysis of the adjuvancy of the CP4 EPSPS protein has also been performed and no similarity to known strong adjuvants is found. Also, bioinformatic analysis does not find it similar to known allergens.

A major point in this assessment of toxicity and allergenicity is that the applicant uses a different form of the protein than the one actually present in the food/feed they want approved: namely the *E.coli* version of the CP4 EPSPS protein and not the authentic plant version of it.

The applicant states that; "There have been a limited number of reports citing oilseed rape flour as an allergen. These studies reported that the four individuals with hypersensitivity to oilseed rape flour worked with animal feed preparation where oilseed rape flour is a component, suggesting the prevalence is low and confined to occupationally exposed populations." (A 5) and further. "The incidence of oilseed rape hypersensitivity in the occupationally exposed population, i.e., scientists or farm workers that handle the plant and the pollen on a daily basis, was 31%, but most of these individuals were hypersensitive to multiple allergens" (A 5).

In section A 5.4 the applicant concludes that they have evidence that the CP4 EPSPS protein is not likely to be allergenic and thus the food (or feed) derived from MON 88302 also is not likely to be more allergenic than other varieties of oil seed rape. This claim is scientifically unjustified and should be rejected.

Thus we must conclude that the applicant does present evidence for potential allergenicity of oil seed rape flour, but does not adress this potential effect for this relevant variety in a scientifically acceptable way. The applicant thus should perform relevant testing of MON 88302 allergenicity potential in animal and human allergenicity testing models.

Recommendation:

- The Applicant should use the plant produced CP4 EPSPS and not the *E.coli* produced CP4 EPSPS to assess the safety of the protein.
- The applicant should adress the issue of potential allergenicity by testing representative feed/food material in animal and human allergenicity testing models.



Feeding experiment

In the dossier presented by the Applicant, no treatment related adverse-effects were observed in animals dosed with CP4 EPSPS protein except from a few minor pathological findings in female mice were observed at necropsy to be randomly distributed among all groups and are commonly seen in the strain of mice used (Harrison et al 2006). Besides, this specific study by Harrison et al is on GT-soybean and has little, if any, relevance to the GT-oil rape in question here. However a number of issues create uncertainty regarding the claim of safety.

First, in this study the *E.coli* produced CP4 EPSPS protein was used to assess the safety of CP4 EPSPS and not the plant-produced proteins. The reason why the applicant prefer the bacterial version is because the levels of introduces proteins in planta are usually too low to allow purification of sufficient quantities for use in safety assessment studies. By using the bacterial version of the protein excludes information on the toxicological potential of the protein in a genetically modified plant. One should always utilize the version present in the plant as mentioned before (Codex work on Foods derived from Biotechnology, CAC/GL 44-2003, p. 14 and 22).

Second, acute oral toxicity studies may detect large effects, yet have little relevance for substances or products which will be fed or consumed over a lifelong period and exhibit chronic effects. Certain toxicological properties will only become evident in case of systematic testing (Spök et al 2004, 2005). With the acute toxicity study, mice and rats are the normal test organisms, but they should also include one non-rodent species (e.g. dogs) for sub-chronic testing. Proper hazard characterization of any effects noted in these studies may require determining mode of action (EFSA, 2008a).

Recommendation:

- The Applicant should use the plant produced CP4 EPSPS and not the *E.coli* produced CP4 EPSPS to assess the safety of the protein.
- The Applicant should include non-rodent species as test organisms for the toxicity studies.
- The Applicant should include a long-term feeding study in the toxicological testing



Environmental risk assessment

The information provided by the applicant substantiates that Europe is the center of origin of oilseed rape, and it gives an important overview of the main oilseed rape producing areas world wide. From this overview presented in table 1. it is evident that the EU countries and Europe as a whole, is the worlds main producing area of oilseed rape seed, oilseed rape oil and oilseed rape meal. The EU production of these commodities constitute 30-45% of the global total. The present EU annual production seed alone from oilseed rape, is estimated at 21.6 million metric tonnes (A 1.2).

In section B 2. the applicant presents detailed figures figures for production and trade, giving oilseed rape seed imports into the EU by country of destination in 2009/2010 season in table 27. This shows imports of 633.000 tonnes annualy to Belgium, 529.000 tonnes to France and 439.000 tonnes to the Netherlands. This is not necessarily evidence of high local use in those countries, but art least for Belgium and the Netherlands probably indicates that these countries harbour the main EU ports of importation of such bulk-material, to be distributed from there within EU. In this context the import figure for Germany of 93.000 tonnes is probably an underestimate, as much of the canola intended for use in Germany is probably imported via Antwerpen or Rotterdam. the table also presents import figures for land-locked European countries such as Austria, which is stated to import 40.000 tonnes of rapeseed annually. Given the substantial quantities and considerable distances and numerous logistical operations necessary for such bulk transport, spillage of viable seed will be unavoidable and we thus must enhance the need for sufficient environmental monitoring plans.

Information about oilseed rape transport and storage

The applicant presents the following referenced and substantiated information: "In general, the oilseed rape in the EU is brought onshore by coasters or inland barges and unloaded to storehouses. From there it is transported to the crushing plant, where it is first cleaned and then pressed in a closed production process" (A 1.4.2) Further in the same chapter the applicant states that; "When rapeseed is imported for use in the oilseed crushing industry it is done in bulk and by shipping boats. While most seed is crushed in or near the ports of entry in the EU, a fraction of the imported viable seed can be transported inland to processing (crushing) facilities by boat, truck or rail (Devos et al., 2011)" (A 1.4.2).

A closer reading of the mentioned source reveals the following additional information; "The particular concerns related to feral GMHT oilseed rape fall within the range of general concerns stated above. They may cause a change in fitness, leading to invasion of seminatural habitats, or to a colonisation of agricultural fields, where additional herbicide applications for weed control may be required due to the unintended stacking of HT traits. Feral GMHT oilseed rape plants may extend the potential for gene flow by acting as stepping stones and by forming populations that accumulate transgenes, thereby contributing to admixtures with commercially grown oilseed rape varieties. Based on such arguments, three



EU Member States invoked national safeguard clause measures to provisionally ban the marketing of specific oilseed rape events on their territory" (Devos et al., 2011).

We fully support this concern of the potential of herbicide tolerant varieties of oilseed rape to escape currently employed transport logistics and establish herbicide tolerant weedy populations, having a competitive advantage due to the presence of genes conferring resistance to the most commonly used herbicides.

Such important questions relate to the importation, storage and within EU transportation of viable seed. These questions should be addressed in the 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.

This application MON 88302 use in food and feed purposes is very specific on several of the questions that such an application formally has to address, while other important questions seem to be given less priority. There is a little mention of the potential for outcrossing, hybridization through drift of pollen from plant resulting from MON 88302 seed accidentally entering the existing agriculture systems. The application presents *B. napus* as primarily self-pollinating with a limited range of pollen drift through wind and insect pollinators. These spatial limitations for horizontal geneflow and hybridization with local varieties are considered sufficient to ensure the safety of the existing European cultivation of *B. napus*. However, several questions remain unresolved, one of them survivability and dissemination of MON 88302 seed from accidental spills. The applicant specifically states that "Dissemination of oilseed rape plants is exclusively by means of seeds, under natural conditions. 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" (C 2.1, p 142).

In the next section the applicant makes a contradictory claim commenting on the issue of "special factors affecting dissemination" stating that "Seed dissemination is increased by excessive pod shattering during harvesting, but seed remains in the area where it is shed" (C 2.1, p 142).

This claim is unsubstantiated and not in accordance with well established scientific knowledge of seed dispersal mediated by birds and other organisms (Howe and Smallwood 1982). Continued traditional agriculture coexistense and implications for such coexistence from avifauna dispersal of viable commercial seed from genetically modified crops has recently been studied in transgenic crops in general (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 Twigg et al estimates the dispersal range of viable B. napus seed as generally less than 10 kilometres in the species of birds investigated (wood duck, Chenonetta jubata), other observations indicate that the potential dispersal range could be much larger in other bird



species. Evidence presented by Hart (2011) 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.

The applicant states that; "Brassica napus is not generally regarded as an environmentally hazardous, colonizing (EC, 2000), or invasive species in undisturbed natural ecosystems (Crawley et al., 2001). Although B. napus has some characteristics typical of weedy species such as a high reproductive capacity, rapid growth and multiple pollination mechanisms (self, wind, insect), it also has many characteristics typical of domesticated species including low genetic diversity, lack of long-distance seed dispersal mechanisms, limited population persistence, lack of primary seed dormancy and an inability to compete well with perennial species (Hall et al., 2005). Brassica napus has been documented to be present in disturbed areas such as roadsides and railways used for transportation of seed and the margins of fields where it has been previously grown" Further, the applicant states that; "Volunteers, including volunteers with herbicide-tolerant traits, can be managed with pre-plant or selective post-emergent herbicide applications or by mechanical means".

We find that the applicant presents information which indicates that the applicant is aware of the highly relevant risk of MON 88302 spillage, contamination, dissemination and growth in the targeted area of receiving environment. we find that the assurances presented by the applicant, that such unwanted presence can be managed by mechanical means or by herbicide application is not sufficient reasurance. In order to protect European environment and agriculture from advantageous presence of MON 88302, a plan for monitoring and remediation is absolutely necessary.

It must also be mentioned that the applicant specifically states that viable whole MON 88302 seed is intended for; "*The import of whole oilseed rape for processing in pet food, in particular seeds for birds*". (A 1.4.2). Production of feed for birds which includes viable MON 88302 seeds is a very serious threat to the European effort to limit the contamination of environment and agriculture by unapproved transgenic cultivars. Few other methods will ensure more efficient and rapid dissemination and establishment of viable volunteers of MON 88302. It is difficult to comprehend how and why the applicant has included this sentence in the application dossier, unless it is either a deliberate plan to influence agriculture coexistense or a lack of fundamental biological and ecological knowledge.

Information on the Environmental Monitoring plan

The applicant describes a plan for case-specific GM plant monitoring (section E 4.3) and general surveillance (E 4.4) but these plans are insufficient to ensure that advantageous presence of MON 88302 in the European environment and agriculture systems be identified and eliminated.

The applicant merely describes existing systems for handling and distribution of seed and produce, including the main European partners involved in this commercial activity. The



backbone of this so-called "Environmental monitoring Plan" seems to be an information service hosted on the internet. There is no plan for surveillance of advantageous presence of MON 88302, nor any plan for testing nor screening of European produce in the exposed areas.

Such an environmental monitoring plan should also take into consideration potential environmental consequences of spillage during import, transportation, storage, handling and processing of MON 88302 within the area of the European Union.

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.

Information on substantial equivalence of the MON 88302 variety

The applicant is expected to describe important compositional changes in the MON 88302 variety, compared to other cultivars.

The applicant presents testing involving 11.900 individual data from comparative assessment of MON 88302 treated with glyphosate as expected in commercial cultivation, comparing the compositional analysis of the cultivar with that of MON 88302 untreated by glyphosate, as well as compositional data from non-transgenic comparators grown in the same environments in parallel plots. The applicant states that there are no meaningful differences in the composition of nutrients, antinutrients and several other measurables. But, importantly the applicant has not analyzed the produce for residues of the pesticides deployed, which must be underlined as being of significant importance.

Experience form application of glyphosate in other transgenic crops such as GT-soy, has demonstrated that glyphosate not only accumulates in these crops in ppm-levels (Duke et al 2003, 2005, Cuhra and Bøhn in prep) but also negatively affects plant metabolism (Zobiole et al 2010, 2011) and can make GT-plants more susceptible to plant pathogens (Huber 2011).

A main issue concerning compositional qualities of GT crops is thus the potential effects on plant, health and environment from the substantial 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 composition of the crops.

Finally, we should mention that the applicant presents the following information, which we find incorrect; "Oilseed rape became widespread as a source of food and animal feed only after 1960 when Canadian scientists made two important genetic modifications to oilseed rape which lead to the first double-low (low-erucic acid and low glucosinolate) variety (Brown et al., 2008)." (A 1.2). We disagree with this use of terminology, claiming similarity



between plant varieties produced by transgenic insertion and varieties evolved by traditional crossing (hybridisation). Despite the fact that it is used in the original source document, the applicant should abstain from repeating a misleading terminology. To avoid misunderstandings and confusion, the term "genetic modification" should be reserved for products from manipulative insertion such as transgenic varieties, but not used for description of varieties produced by non-disruptive methods used for centuries in traditional agronomy. The latter methods are largely acknowledged as conserving the host genome and we see no scientific argumentation to support an assumption of equivalence between these fundamentally different approaches.

Recommendation:

- The applicant should present a Environmental Monitoring Plan according to accepted standards.
- The applicant should ensure that viable seed of MON 88302 is not used in commercial bird-feed for the European market.
- The applicant should take measures to ensure future coexistense of non-transgenic cultivars by eliminating the risk of both accidental spillage and contamination of transport equipment.



<u>Missing or insufficient information in relation to requirements under the Norwegian</u> <u>Gene Technology Act</u>

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 MON88302. 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 MON88302 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 MON88302 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 LMO, 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 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 MON88203. 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 MON88203 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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