GenØk - Centre for Biosafety

Vår ref:2013/H108
Deres ref: 2013/7562

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 20.11.13

Vedlagt er innspill fra GenØk – Senter for biosikkerhet om høringen EFSA/GMO/NL/2012/108 for MON 87708 × MON 89788 som gjelder mat, fòr, import og prosessering av genmodifisert soya fra Monsanto Company.

Hvis du har noe spørsmål, vennligst ta kontakt.

Med vennlig hilsen,

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GenØk - Centre for Biosafety

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Assessment of the technical dossier submitted under EFSA/GMO/NL/2012/108 for approval of transgenic soy, MON 87708 x MON 89788, Monsanto Company

Submitted to

Miljødirektoratet

By

Lise Nordgård, Idun Merete Grønsberg, Marek Cuhra, Marianne Iversen, Rosa A. Binimelis

Centre for Biosafety – GenØk
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KONKLUSJON PÅ NORSK

Vi trekker frem mangler i dossieret som ikke gir grunnlag for en konklusjon om sikker bruk, samfunnsnytten og bidrag til bærekraftighet av soyaplanten MON 87708 × MON 89788.

Hovedkonklusjon og anbefalinger

GenØk – Senter for Biosikkerhet viser til brev fra Miljødirektoratet angående høring av søknad **EFSA/GMO/NL/2012/108** som omfatter soyaplanten MON 87708 × MON 89788 for bruksområdene import, prosessering, mat og fòr.

Soyaplanten MON 87708 × MON 89788 er en stablet hybrid med to ulike gener satt inn som ifølge søker gir plantene økt toleranse mot glyfosat og endret fettsyresammensetning i frøene.

Stablede planter har generelt en mer kompleks genetisk sammensetning og derfor større potensial for opp- og nedregulering av plantens egne gener. Derfor burde de gjennomgå grundig testing før eventuell markedsadgang. GenØk mener det ikke er faglig velbegrunnet å godkjenne den stablede planten basert på at foreldrelinjene, hver for seg, er godkjent.

CP4 EPSPS-proteinet gjør soyaplantene tolerante overfor ugrasmidler med virkestoffet glyfosat. I den senere tid har laboratorieforsøk 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.

Dmo-proteinet gjør soya plantene tolerante overfor ugrasmidler med virkestoffet dicamba. Dicamba har vært betegnet som et plantevernmiddel med lav toksisitet. I den senere tid har det vært publisert artikler som indikerer indirekte negative effekter av dicamba på insekter. Søker bør derfor undersøke nærmere potensielle miljø- og helsemessige effekter

Produsenten har ikke adressert viktige helseaspekter ved introdusering av MON 87708 × MON 89788 i matkjeden.

GenØk mener at den molekylære beskrivelsen av MON 87708 × MON 89788 er utilstrekkelig for at man kan utelukke nye uønskede effekter som kan utøve en risiko for konsumentens helse eller for miljøet.

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 soyaplanten MON 87708 × MON 89788.

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 soyaplanten MON 87708 × MON 89788.

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av soyaplanten MON 87708 × MON 89788 i de bruksområder det søkes om.

SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED EFSA/GMO/NL/2012/108

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 MON 87708 × MON 89788 , 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 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.

Key findings

After an analysis of many of the portions of the dossier of MON 87708 × MON 89788 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 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 regulator is encouraged to ask the Applicant to provide direct evidence of the lack of combinatorial effects arising from the expression of the stacked proteins in the plant, instead of relying on the assessment of non-harm of the target genes existing independently, before a conclusion of safety can be scientifically justified.
- The regulator is encouraged to ask the Applicant to address potential environmental consequences and combinatorial effects by using multiple herbicides/pesticides on the same plant.
- The regulator is encouraged to ask the Applicant to address the potential influence of dicamba on food-web dynamics.
- The regulator is encouraged to ask the Applicant to address potential environmental consequences and combinatorial effects by using multiple herbicides on the same plant.
- 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.
- The regulator is encouraged to ask the Applicant to comment on the fate of potential herbicide residues.
- The Applicant should provide additional data using comprehensive set of smaller probes in order to evaluate the genetic stability of the event.
- The Applicant should provide the electropherograms for the sequence analysis in order to be able to check the quality of the sequencing.
- The Applicant should provide evidence that the antibodies used in the protein characterization would detect all novel in-planta produced isoforms.
- The Applicant should provide data to substantiate claims of specificity; either by using the in-planta produced proteins or by demonstrating equivalence between the test protein and the in-planta produced form.
- The Applicant should supply evidence about the substrate specificity of DMO by testing substances more relevant to the safety assessment, using the in-planta produced DMO proteins.
- The Applicant should use plant version of the protein(s).
- The Applicant should include a chapter on identification of the transgenic proteins in the stack and not base conclusion of analysis made in single parental lines.
- The Applicant should perform analysis on the combined event (MON87708 x MON87798) and base conclusions on that rather than on the single events separately.
- The Applicant should perform repeated dose studies for analysis of transgenic proteins in combination for analysis of toxicological potential.
- The Applicant should provide data on the glycosylation status of the proteins to the allergenic risk assessment.
- The regulator is encouraged to ask the Applicant to submit required information on the social utility of MON 87708 × MON 89788 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 and contribution to sustainable development of **MON 87708 × MON 89788**. **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 MON 87708 × MON 89788, 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/2012/108

About the event

According to the developer, MON 87708 × MON 89788 was obtained by traditional breeding of two parental lines; one derived from MON87708 and the other one derived from MON89788. However, genetic modification has been used in the development of each of the parental lines through *Agrobacterium* mediated transformation of soybean tissues.

MON87708 contains a gene derived from *Stenotrophomomas maltophilia* (*S.maltophilia*) that expresses DMO, a mono-deoxygenase enzyme that rapidly demethylates dicamba rendering it inactive, thereby conferring tolerance to dicamba herbicide.

MON89788 contains a gene derived from *Agrobacterium* sp. strain CP4 (*cp4epsps*) that expresses CP4 EPSPS protein conferring tolerance to glyphosate herbicide.

The use of MON 87708 × MON 89788 will enable growers to utilize both dicamba and glyphosate for effective control of weeds.

The scope of the application is for food, feed, processing and import with the exception of cultivation.

Assessment findings

Assumptions-based reasoning on stacked events

Until recently, the dossiers submitted for marked authorization almost only covered single GM events. Today there is a clear trend to combine two or more transgenic traits present in single events through traditional breeding. However, information on how these GM stacked events should be assessed is limited and in some cases assessment data for each single GM events has been taken into account to prove the safety of the whole food/feed.

Stacked events are in general more complex and it has been an increased interest in the possible combinatorial and/or synergistic effects that may produce unintended and undesirable changes in the plant – like the potential for up- and down regulation of the plants own genes. Interactions with stacked traits cannot be excluded that the group of expressed toxins in the plant can give specific immunological effects or adjuvant effects in mammals (Halpin 2005, DeSchrijver et al 2006). Then (2009) reviews and discusses the evidence for changes in activity and specificity of Bt proteins dependent on synergistic interactions with extrinsic features. Such changes may critically influence the bioactivity and hence the potential for unintended effects. This is why combinatorial, synergistic effects must be carefully considered in the development and risk assessments of stacked events and robust data are necessary to identify whether the combined presence of transgenes influences expression levels, e.g. by silencing effects.

Most of the information submitted in this safety assessment is derived from previous finding with the single lines. In general the applicant describes most of the traits and characteristics of

the "stacked event" as being the same as those of the parental GM events used in production of GM maize.

The applicant has not demonstrated that interactions among the different transgenic proteins, particularly for allergenic or toxic effects, are not taking place in this event, despite evidence of the potential (Mesnage et al 2012). Assumptions-based reasoning with single events should not replace scientific testing of hypotheses regarding interactions. GenØk means that stacked events cannot be approved based on the information on the single events.

Recommendation:

- The regulator is encouraged to ask the Applicant to provide direct evidence of the lack of combinatorial effects arising from the expression of the stacked proteins in the plant, instead of relying on the assessment of non-harm of the target genes existing independently, before a conclusion of safety can be scientifically justified.
- The regulator is encouraged to ask the Applicant to address potential environmental consequences and combinatorial effects by using multiple herbicides/pesticides on the same plant.

Herbicides

Glyphosate

Event MON 87708 × MON 89788 expresses a *CP4EPSPS* gene from *Agrobacterium sp. line CP4* that confers tolerance to herbicides products containing glyphosate.

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, Chura 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 2003). In a recently published study by Seralini et al (Seralini et al 2012) the authors concludes that long term exposure of complete agricultural glyphosate herbicide formulations, at concentrations well below officially set safety limits, induce severe hormone-dependent mammary, hepatic and kidney disturbances in rats.

Dicamba

Event MON 87708 × MON 89788 expresses a *dmo* gene that confer tolerance to herbicide products containing dicamba. Dicamba is a benzoic acid herbicide that mimics the plant hormone auxin, causing uncontrolled growth which eventually kills plants.

Dicamba is considered as an herbicide with low toxicity, but with high residuality. In recent years dicamba has received more risk-related attention due to the on-going evolution of glyphosate resistance in weed species and use of other agrochemicals in some agroecosystems (Binimelis et al 2009, Ensminger et al 2013). A recent article has been published indicating indirect negative effects of dicamba on insects, while highlighting that little research has been conducted on the issue to date in despite dicamba is, along with 2,4-D, causing most herbicide-drift damage to non-target plants even though present limited agricultural usage (Love et al 2011, Bohnenblust et al., 2013).

Recommendation:

- The regulator is encouraged to ask the Applicant to address the potential influence of dicamba on food-web dynamics.
- The regulator is encouraged to ask the Applicant to address potential environmental consequences and combinatorial effects by using multiple herbicides on the same plant.
- 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.

Herbicide residues

The Applicants does not comment on the fate of potential herbicide residues that might be converted into other compounds when processed. That MON87708 x MON89788 is equal to conventional varieties in it untreated form might be true, however an important point here is that the genetic modification allow for heavy treatments with herbicides glyphosate and dicamba, and potential effects of processing of potential residues (the dossier provides no estimation of expected herbicide residues) of these should be included in the evaluation of the safety of MON87708 x MON89788. A toxicological test with soybean processed products is justified.

This comment also applies to section B in general and p.141 *3.7 Effects on human and animal health*, which also ignores potential effects of exposure to the herbicides and metabolites thereof.

Recommendation:

- The regulator is encouraged to ask the Applicant to comment on the fate of potential herbicide residues.

2 Molecular characterizations (p. 17)

2.1 Information relating to the genetic modification (p. 17)

The Applicant states that ”*the data on molecular characterization did not identify features of MON 87708 × MON 89788 with a potential to raise any safety concerns*”. However, most of the information submitted in this safety assessment is derived from previous finding with the single lines and not with the actual event.

In the Application for the MON 87708 event (EFSA/GMO/NL/2011/93*)*

GenØk submitted a hearing to the Norwegian Environment Agency in June 2011 regarding MON 87708 event (EFSA/GMO/NL/2011/93). Based on our findings, we proposed a number of specific recommendations, summarized here and detailed in our letter regarding EFSA/GMO/NL/2011/93.

Recommendations
The Norwegian Environment Agency is encouraged to request the following:
1. The Applicant should be required to provide a post-release plan that provides certainty to the regulator on:

a. intended and maximum levels of dicamba applications per season per locality;
b. ability of the Applicant or adopters of dicamba-tolerant soybeans to detect the emergence of dicamba-tolerant weeds with a sensitivity that would allow them to be controlled without resort to higher levels of dicamba application or alternative herbicides.

2. The Applicant should provide information on

c. intended and possible maximum dicamba residues on dicamba-tolerant plant materials at various stages in the production chain;
d. intended and possible maximum dicamba metabolite residues on dicambatolerant plant materials at various stages in the production chain;
e. non-target effects on microorganisms including those that could select for cross-resistance to clinical or veterinary antibiotics at possible maximum frequencies and doses of application;
f. effects on nitrogen-fixing microorganisms at both intended and possible maximum dicamba application levels.

3. The Applicant should supply evidence about the substrate specificity of DMO by testing substances more relevant to the safety assessment, using the in-planta produced DMO proteins.

4. The Applicant should be required to submit data from field trials covering more than one field season in order to allow adequate exposure to the variety of conditions met in nature (Codex, 2003).

5. The Applicant should the clarify functional status of the transgenic protein after processing with properly designed experiments, and further test the effects of MON 87708 inhalation in

animals that are used as models of acute respiratory syndrome, compared with inhalation of the proper conventional comparator. This should include an analysis of allergenicity and toxicity.

6. The Applicant should be requested to investigate the differences in composition that may be directly attributed to the treatment with dicamba and the relevance of these for the risk assessment.

7. The Norwegian Environment Agency should request data from proper immunostimulation and allergenicity testing of MON 87708 including tests from diet and inhalation exposures.

8. The Applicant should report the DMO concentration of feed used in the feeding trials at the beginning and the end of the studies.

9. The Applicant should provide feeding data obtained with MON 87708 that has been grown under the relevant agronomic conditions, i.e. in the presence of dicamba.

10. The Applicant should provide evidence that the effect of MON 87708 on spleen parameters in the rat feeding study was indeed incidental or experimentally determine the cause for the variation in spleen size of female rats fed with 15% MON 87708.

11. The Applicant should provide evidence that the antibodies used in the protein characterization would detect all novel in-planta produced isoforms.

12. The Applicant should report detection limits for all methods.

13. The Applicant should comply with EFSA and Codex guidelines and provide evidence that all isoforms of the newly expressed proteins are not posttranslationally modified.

14. The Applicant should provide data to substantiate claims of specificity, either by using the in-planta produced proteins or by demonstrating equivalence between the test protein and the in-planta produced form.

15. Given the deletion and insertions 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).

16. The Applicant should submit required information on the social utility of MON 87708 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act

In the Application for the MON 89788 event (EFSA/GMO/NL/2006/36)

GenØk have not previously submitted a hearing regarding MON 89788 event (EFSA/GMO/NL/2006/36) to the Norwegian Environment Agency.

In the Application for the MON 87708 × MON 89788 event (EFSA/GMO/NL/2012/108)

2.2.2. Information on the sequences actually inserted/deleted or altered (p.25)

Comments and recommendations on Southern blots that was done to verify the presence of MON 87708 in MON 87708 × MON 89788 (*Section 2.2.2 i*)

- The sizes of the used probes are considered too long and they 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 (probe) and the matching recombinant DNA (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.

- The southern blot picture lacks a labeled size marker. A marker should always be present in order to check if the expected sizes are correct.

Comments and recommendations on Southern blots that was done to verify the presence of MON 89788 in MON 87708 × MON 89788 (*Section 2.2.2 i*)

- Only two probes were used in the southern blot studies: the first one with 1,1kb and the second one with 1,6kb. No probes to check backbone DNA were used.
- The sizes of the used probes are considered too long and they 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 (probe) and the matching recombinant DNA (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.
- In the application for the MON 89788 event (2006), seven probes covering the whole inserted DNA were used and also three probes to check backbone DNA.
- The probes used in this application were Probe 5 and Probe 6 used in the Application EFSA-GMO-NL-2006-36 Monsanto Company (2006).
- The southern blot picture lacks a labeled size marker. A marker should always be present in order to check if the expected sizes are correct.
- Both long- and short runs should have been performed to allow the resolution of high molecular weight fragments and of smaller molecular size bands.

Comments and recommendations on organization and sequence of the inserted genetic material at each insertion site *(Section 2.2.2 ii, p.25)*

MON87708

- All the information about organization and sequence of this new GM are the same as the one used the Application EFSA/GMO/NL/2011/93 Monsanto Company.
- The Applicant claims that (p.35) *"Since the inserts present in MON 87708 × MON 89788 correspond to those of the parental lines, the characteristics of the insertions and the 5' and 3' flanking sequences should be conserved in this combined-trait product*". However, the analysis at the insertion site of MON 87708 identified a 899 bp deletion (and a 128 bp insertion just 5`of T-DNA I, and a 35 bp insertion just 3` of T-DNA I). The Applicant claims that minor deletions and/or insertions are not uncommon in this process. However:
- The Applicant does not give the sequence of the internal primers used for sequencing.
- The Applicant does not show the electropherograms to check the quality of the sequencing.
- Monsanto Genomics Sequencing Center using dye-terminator chemistry are performing the sequencing reaction, however an independent laboratory should be used.

MON89788

- The Applicant does not give the sequence of the internal primers used for sequencing.
- The Applicant does not show the electropherograms to check the quality of the sequencing.
- Monsanto Genomics Sequencing Center using dye-terminator chemistry are performing the sequencing reaction, however an independent laboratory should be used
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Recommendation:

- The Applicant should provide additional data using comprehensive set of smaller probes in order to evaluate the genetic stability of the event.
- The Applicant should provide the electropherograms for the sequence analysis in order to be able to check the quality of the sequencing.

2.2.3 Information on the expression of the inserted/modified sequence (p.39)

Assessment of the newly expressed protein

Levels of DMO and CP4-EPSPS were analyzed in a field trial by ELISA in both dicamba and glyphosate treated and untreated tissue samples (forage and seed) at representative growth stages. The detected protein expression levels of Cp4-EPSPS and DMO were considered as low and there were no big differences in expression levels between the parental lines and the stack MON 87708 X MON 89788, others than in forage, where the level of CP4-EPSPS was lower in the stack than in the parental line. The Applicant does not discuss this difference further.
The low level of protein is not considered to cause any harm when used in food/feed.
Also, as the assessments of this stacked event are based on previous single assessed events, the Applicant should have included a chapter on the identity of the transgenic proteins and the equivalence to the microbial versions as these are the ones used in the previous assessment of the single parental lines. This could have been done with methods for molecular weight characterization or using immunoblot for verification, MALDI-TOF MS or else. If this had been performed, one could have verified the size of the newly expressed proteins, and if they reacted with the corresponding antibodies. SDS-PAGE is however used to look at stability of the transgenic proteins under pH and temperature influences, and not in this part.

We have previously commented on the expression of DMO in MON87708 (EFSA-GMO-NL/2011-9) and these recommendations also account for the stacked event MON 87708 X MON 89788:

Recommendation:

- The Applicant should provide evidence that the antibodies used in the protein characterization would detect all novel in-planta produced isoforms.
- The Applicant should provide data to substantiate claims of specificity; either by using the in-planta produced proteins or by demonstrating equivalence between the test protein and the in-planta produced form.
- The Applicant should supply evidence about the substrate specificity of DMO by testing substances more relevant to the safety assessment, using the in-planta produced DMO proteins.

Toxicity
The proteins DMO and CP4-EPSPS are expressed in the plant in combination. However, the safety characterization is based on evaluation of the two proteins separately (EFSA-GMO-NL-2011-93, EFSA-GMO-NL2006-36).
For the toxicological assessment of DMO and CP4-EPSPS, the history of safe use, no structural similarity to known toxins or biologically active proteins, no acute toxicity effect in mammals and large margin of exposure terms, are used to conclude for the safety. However, it is recommended that the Applicant use the real plant versions of the proteins for the safety

assessments as plants and bacteria differ in their post-translational processing of proteins. This should be considered and further analysed.
Testing of the whole GM food/feed is not considered as needed due to the evidenced "no adverse effect on human or animal health" and the demonstrated safety ("safe as conventional soy bean") for the single, parental events. The safety assessments are based on previous assessments of the single parental lines and not for the stack the Applicant seeks approval for. We recommend that the Applicant performs a safety assessment based on the combination of the proteins in the stack, as this will be more real and also reveal potential combinatorial effects.
Repeated dose toxicity studies are also not considered as necessary based on the demonstrated safety of the DMO and EPSPS proteins in previous assessments on the single parental lines. Again, we recommend that the proteins in question are checked in combination studies.
The DMO and CP4-EPSPS proteins have apparent separate mechanisms of action and are not thought to interfere. This is however not analyzed by the Applicant.
"Vitenskapsgruppen for Mattrygghet" (VKM)(VKM Dok 11-309) has previously commented that the Applicant of the MON87708 event should have provided a 90 day repeated dose study and a 42 days feeding experiment with feed containing the protein(s) in question together with herbicide treatment to provide data for evaluation of potential toxic effects. We support this suggestion and add that this has not been performed for the stacked event containing MON87708, either. We therefore recommend that this is done to reveal potential combinatorial effects of the proteins in the stack MON 87708 XMON 89788.

Allergenicity
A weight of evidence approach is used for the assessment of the DMO and CP4-EPSPS proteins in the MON 89788 X MON 87708. Here the following issues are considered: "Proteins obtained from non-allergenic source, constitutes small part of the total protein, lack of structural similarity to known allergens and rapid digestion in SGF". These assessments are based on previous assessments of the single parental lines and not the stack as a whole. Also, the previous assessments are based on microbial versions of the proteins and not the plant version. Also, the Applicant says that the stack "has the same potential as conventional soy" and that the potential is "inherited in MON 87708X MON 89788. This is however not checked, but as assumed.

Specific serum screening
Specific serum screening is performed in assays from the single parental lines and not from the stack. That is: not for the combination of the stacked proteins. Thus this is not considered as necessary based on the evidence provided by the Applicant. We still think a screening with the transgenic proteins in a combined assay would have given a more exact answer to this issue of allergenicity towards the stack MON 88708 X MON 89788.
We have previously commented upon the allergenicity assessment in MON87708 (EFSA-GMO-NL/2011-9) where we recommended that data for proper immunestimulation and allergenicity testing of MON87708 including tests from diet and inhalation exposures were provided. We can add that this also includes a proper testing of the allergenic potential of the stack MON 89788 X MON 87708.
The Applicant does not mention the glycosylation status of the transgenic proteins in the stack MON 88708 X MON 89788 related to the allergenic potential. This should have been part of the assessment of the allergenic potential of the stack.

Recommendation:

- The Applicant should use plant version of the protein(s).
- The Applicant should include a chapter on identification of the transgenic proteins in the stack and not base conclusion of analysis made in single parental lines.
- The Applicant should perform analysis on the combined event (MON 87708 x MON 87798) and base conclusions on that rather than on the single events separately.
- The Applicant should perform repeated dose studies for analysis of transgenic proteins in combination for analysis of toxicological potential.
- The Applicant should provide data on the glycosylation status of the proteins to the allergenic risk assessment.

Social utility, ethical 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 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.

In this case, the Applicant states that applications for the full range of uses (including cultivation) will be made in Argentina, Brazil, Canada and Japan. Although the literature concerning the socio-economic aspects related to the cultivation of GM soybeans in these producing countries is extense, the Applicant does not mention any these references, nor there is an attempt to identify how MON 87708 × MON 89788 soybean might contribute to sustainability and social utility (neither in the producing countries nor in Norway or Europe).

On the contrary, a recent article by Leguizamón (2013) analysing the contribution of GM soy from the socio-economic (i.e. labour and rural depopulation, agricultural deskilling, distribution of land, protection of indigenous and small peasant communities, increase of violence related to landgrabs, herbicide-sprays over rural populations or food sovereignty) and environmental perspectives (i.e. expansion of the agrocultural frontier, deforestation, biodiversity, nutrient depletion and soil structure degradation), concludes that although the massive adoption of GM soy has provided important economic revenues, "*the GM soy-based agro-export model as currently configured in Argentina is a socially and ecologically unsustainable model of national development*". Although there is an important controversy, similar conclusions are also reached by other authors for the case of Argentina or Brazil (see e.g. Ortega et al., 2005, Pengue, 2005; Binimelis et al., 2009, Richards, 2010, Richards, 2010, Catacora-Vargas, 2012; Catacora-Vargas et al., 2012).

Therefore, 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 MON 87708 × MON 89788 soybeans.

On the sustainability of the product and co-technology, MON 87708 × MON 89788 confers soybeans tolerance to herbicides containing glyphosate and dicamba. The increased use of glyphosate in countries with a massive adoption of herbicide-tolerant GMOs is associated with the appearance of a growing number of tolerant or resistant weeds, with socio-environmental consequences apart from the loss of productivity (see e.g. Powles, 2008, Green, 2009). As a response, new genetically modified crops that allow the use of yet more herbicide are introduced, in turn reinforcing the emergence (and spread) of herbicide-resistant weeds. This intensification process, known as "transgenic treadmill", has been documented in countries where the MON 87708 × MON 89788 soybean is planned to be introduced, such as Argentina (Binimelis et al., 2009).

In fact, although it is expected that the tolerance to dicamba modification could provide an alternative for controlling weeds in glyphosate-tolerant soybean fields and for extending the effective lifetime of glyphosate (Behrens et al., 2007), the effectiveness of dicamba for controlling weeds such as waterhemp is lower than glyphosate, which could add to the "treadmill" effect for farmers if control is low. Besides, reduced sensitivity to dicamba has also been recently reported in *Amaranthus* species by Bernard et al (2012), which makes the authors conclude that "*The commercialization of soybean, cotton, and corn resistant to 2,4-D and dicamba should be accompanied by mandatory stewardship practices that will minimize the selection pressure imposed on other waterhemp populations to evolve resistance to the synthetic auxin herbicides*". Moreover, the contribution of this strategy for sustainability has also been questioned. In a recent article on weed management, Mortensen et al (2012) indicate: "*In response to the outbreak of glyphosate-resistant weeds, the seed and agrichemical industries are developing crops that are genetically modified to have combined resistance to glyphosate and synthetic auxin herbicides. This technology will allow these herbicides to be used over vastly expanded areas and will likely create three interrelated challenges for sustainable weed management. First, crops with stacked herbicide resistance are likely to increase the severity of resistant weeds. Second, these crops will facilitate a significant increase in herbicide use, with potential negative consequences for environmental quality. Finally, the short-term fix provided by the new traits will encourage continued neglect of public research and extension in integrated weed management.*"

As this application excludes the cultivation of MON 87708 × MON 89788 in the EU, the risk assessment is only focused on the import, processing and all other uses but does not assess the cultivation phases, and the potential impacts in the producing countries. However, the Gene Technology Act applies not only for Norway but also for cultivating countries, and therefore, information for the risk assessment on the cultivation, management and harvesting stages (as well as the post market environmental monitoring) is required in order to assess the sustainability criteria laid down in the Act. The Applicant has not provided information on how long (e.g. number of planting seasons) it will take before the MON 87708 × MON 89788 containing plants develop sensitivity to the combined glyphosate and dicamba herbicides.

Therefore, it would be incongruent with the principle of sustainable development. The Applicant should thereby provide the necessary data in order to conduct a thorough assessment on these issues. It is also important to evaluate whether alternative options (e.g. the parental non-GM version of this MON 87708 × MON 89788) may achieve the same outcomes in a safer and ethically justified way.

Recommendation:
• The regulator is encouraged to ask the Applicant to submit required information on the social utility of MON 87708 × MON 89788 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.

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 MON 87708 × MON 89788. 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 MON 87708 × MON 89788 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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