GenØk - Centre for Biosafety

Vår ref:2013/H100
Deres ref: 2012/16061 ART-BI-DHT

Direktoratet for naturforvaltning
Tungasletta 2
7485 Trondheim
Dato: 03.01.13

Vedlagt er inspill fra GenØk – Senter for Biosikkerhet om høringen EFSA/GMO/NL/2011/100 for MON 87705x89788 fra Monsanto Company

Vennligst ta kontakt hvis du har noen spørsmål.

Med vennlig hilsen,

Lise Nordgård
Forsker
GenØk – Senter for Biosikkerhet
lise.nordgard@uit.no

Bidragsytere:

Idun Merete Grønsberg
Forsker
GenØk – Senter for Biosikkerhet

Arinze Stanley Okoli
Forsker
GenØk – Senter for Biosikkerhet

Vinicius Vilperte
Master student
GenØk – Senter for Biosikkerhet

GenØk - Centre for Biosafety

Vår ref:2013/H100
Deres ref: 2012/16061 ART-BI-DHT

Assessment of the technical dossier submitted under EFSA/GMO/NL/2011/100 for approval of transgenic soy, MON 87705 x 89788, Monsanto Company

Submitted to

Direktoratet for Naturforvaltning

By

Lise Nordgård, Idun Merete Grønsberg, Arinze Okoli, Vinicius Vilperte

Centre for Biosafety – GenØk
January 2013

KONKLUSJON PÅ NORSK

Vi trekker frem mangler i dossieret som ikke gir grunnlag for en konklusjon om sikker bruk, samfunnsnytten og bidrag til bærekraftighet soyaplanten MON87705xMON89788.

Hovedkonklusjon og anbefalinger

Genøk –Senter for Biosikkerhet viser til brev fra Direktoratet for naturforvaltning (DN) angående høring av søknad EFSA/GMO/NL/2011/100 som omfatter soyaplanten MON87705xMON89788 for bruksområdene import, prosessering, mat og fòr. Soyaplanten MON87705xMON89788 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 stablede planter 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 laboratorie forsøk vist at glyfosat kan føre til celleskader, blant annet i humane embryoceller. Undersøkelser har også vist en skadelig effekt på vassdrag og vannorganismer. I tillegg forstyrrer glyfosat næringsstoffomsetninga i jord.

Produsenten har ikke adressert viktige helseaspekter ved introdusering av MON87705xMON89788 i matkjeden. Det er ikke fremvist tilstrekkelig dokumentasjon på at de nye dsRNA uttrykt i soya MON87705xMON89788 ikke har andre utilsiktede effekter på andre genutrykk eller at det ikke oppstår andre metabolske forandringer. Det er oppsiktsvekkende og av stor betydning at produsenten ikke har undersøkt og utelukket at det er et lavt-utrykk av små peptider fra det introduserte dsRNAet. Søker har bare argumentert for at slike peptider ikke er tilstede uten at det er fremlagt vitenskapelige bevis for dette. Genøk mener dette viser at den molekylære beskrivelsen av MON87705xMON89788 er utilstrekkelig for at man kan utelukke nye proteinbaserte uønskede effekter som kan utøve en risiko for konsumentens helse eller for miljøet.

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 MON87705xMON89788

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 MON87705xMON89788.

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av soyaplanten MON87705xMON89788 i de bruksområder det søkes om.

SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED EFSA/GMO/NL/2011/100

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 MON87705xMON89788, 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.

This submission was built in large part using the **Biosafety Assessment Tool** (https://bat.genok.org/bat/) produced by the University of Canterbury and GenØk – 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 technical dossier "EFSA/GMO/NL/2011/100", submitted by the Applicant.

Key findings

After an analysis of many of the portions of the dossier of MON87705xMON89788 submitted by the Applicant, we outline a number of inadequacies in the information submitted that do not justify the Applicant's conclusion of safety. Our input focuses on a critique of the Applicant's dossier and covers two issues:

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

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

Recommendations

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

The Direktoratet for naturforvaltning is encouraged to request the following:

1. The regulators are encouraged to fill the research gaps
2. The Applicant should demonstrate the lack of interactive effects between transgenic proteins through proper scientific testing and evidence gathering, rather than justify the lack of testing based on assumptions-based reasoning of no effects.
3. Most of the information submitted in this safety assessment is derived from previous finding with the single lines. Stacked events should not be approved based on the information on the single events but on the actual event.
4. Clearly MON 87705 contains already the *cp4 epsps* gene; thus, the need for the present stacked event is merely for an increased expression level of the protein. The Applicant should thus provide good scientific evidence to justify the safety of the expected increased dietary intake of CP4 EPSPS. The data provided in sections 3 & 4 lack relevant scientific rigors.
5. The stacked event of MON 87705 x MON 89788 does increase the level of CP4 EPSPS; however, it does not add any value to the food because the fatty acid quality remains unaffected. Given that MON 87705 has already been approved, there is no intuitive reason to approve a stacked event that merely increases the level of non-essential enzyme thus increasing the level of health risks. Besides, the application is not for cultivation, thus, the EU does not need an event with increased resistance to glyphosate. This should be explained by the Applicant.
6. The Applicant should provide data, for further examination, on the unintended effects on the plants of increased expression of the CP4 EPSPS proteins, which potentially can have implications on metabolite expressions by the plants, some of which can be anti-nutrients or toxins.
7. The Applicant should identify or analyze off-target effects of the novel dsRNAs expressed in soybean MON87705xMON89788, or other unintended metabolic changes.
8. When a small RNA molecule will or might not act as a gene regulator is not always known in advance. Therefore, it cannot be assumed that novel small RNAs that might be created in MON87705xMON89788 will likewise be safe but should be tested and demonstrated to be safe.

9. The Applicant should submit required information on the social utility of MON87705xMON89788 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 MON87705xMON89788. **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 MON87705xMON89788, 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/2011/100

About the event

According to the developer, MON87705xMON89788 has been genetically modified to provide tolerance to glyphosate by expressing the EPSPS protein and to selectively down-regulate two key enzymes, FATB and FAD2, involved in the soybean seed fatty acid biosynthetic pathway. The aim of this crossing is described as to make soy that is "healthy, lowering cholesterol and LDL-L concentrations". Thus, this soy is announced as a healthier alternative to the others at present. They also say that this soy will produce oil that is more favorable for use in the industry/processing.

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. That 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 Applicant should demonstrate the lack of interactive effects between transgenic proteins through proper scientific testing and evidence gathering, rather than justify the lack of testing based on assumptions-based reasoning of no effects.

Glyphosate tolerance

Event MON87705xMON89788 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, Cuhra et al 2012). Studies in animals and cell cultures indicate possible health effects in rodents, fish and humans. Glyphosate given in the feed to pregnant female rats resulted in higher embryonic mortality and aberrations in the skeleton (Dallegrave et al. 2003). Nile-tilapia (Oreochromis niloticus) fed sublethal concentration of Roundup (active ingredient: glyphosate) resulted in a number of different histopathological changes in organs (Jiraungkoorskul et al. 2003). Experiments with sea urchins exposed to Roundup influenced early cell divisions (Marc et al 2002), effects that have relevance to potential health effects in many eukaryotic organisms, including domestic animals and humans. Exposure to Roundup affected the CDK1/CyclinB regulator which is nearly identical in sea urchins and humans.

Glyphosate has also been shown to negatively affect the differentiation of nerve cells (Axelrad et al 2003). In human placenta cells, Roundup is more toxic than the active ingredient glyphosate (Richard et al 2005). The authors concluded that additional components of Roundup increase the biological availability and accumulation in organisms.

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

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

Unintended potential adverse effects derived from the intended modification for dsRNA – mediated silencing

The modification of MON87705, in MON87705xMON89788, is based on dsRNA silencing to selectively down-regulate two key enzymes involved in the soybean seed fatty acid biosynthetic pathway. This is a type of manipulation that has not benefited from human food safety studies to our knowledge (Heinemann 2009).

The applicant claims that "*dsRNAs are found commonly in eukaryotes, including plants, for endogenous gene suppression and are composed of nucleic acids. Nucleic acids have a long history of safe consumption and are considered generally recognized as safe (GRAS) by the US FDA. There is no evidence to suggest dietary consumption of RNA is associated with mammalian toxicity or allergenicity*". The assertion is incorrect in saying that because the effects of dsRNA are sequence-specific and the Applicant has provided no evidence that the transgenic dsRNA has ever been consumed by humans (or wild vertebrate and invertebrate animals) or consumed in prepared foods. A history of consuming small RNA molecules in plants is not the same as extrapolating the safety of all small RNA molecules. When a small RNA molecule will or might not act as a gene regulator is not always known in advance (BAT). Therefore, it cannot be assumed that novel small RNAs that might be created in MON87705xMON89788 will likewise be safe

From the literature, it is clear that dsRNA can have significant biological impact. Recent research (Zhang et al 2012, CERA 2011, Baum et al 2007, Gordon and Waterhouse 2007, Mao et al 2007) establishes beyond doubt that novel RNAs of recombinant or synthetic origin cannot be "generally regarded as safe" but must be tested and demonstrated to be safe when consumers or wildlife is exposed through food or inhalation.

Recommendation: When a small RNA molecule will or might not act as a gene regulator is not always known in advance. Therefore, it cannot be assumed that novel small RNAs that might be created in MON87705xMON89788 will likewise be safe but should be tested and demonstrated to be safe.

Molecular characterization

The Applicant states that "*the data on molecular characterization did not identify features of MON87705xMON89788 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.

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

Comments and recommendations on Southern blots that was done to verify the presence of MON 87705 in MON87705xMON89788 (*Section A2.2.2 i*)

- Only two probes were used in the southern blot studies: the first probe (1,8kb) spanning the T-DNA I and the second one (1,1kb) spanning T-DNA II. 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 87705 event (2010), six probes covering the whole insert were used and also four probes to check backbone DNA.
- The probes used in this application were the Probe 1 and Probe 6 used in Application EFSA-GMO-NL-2010-78 Monsanto Company (2010).
- 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.
- According the application **(p.29)** "*PV-GMPQ/HT4404 digested with Xho I/Nco I produced a single band at ~9.9 kb (Figure 3, lane 1), which indicates that the probes hybridized to their corresponding sequences in the plasmid vector. This expected band at ~9.9 kb co-migrated with a ~10 kb endogenous band, resulting in a more intense signal*." 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 Southern blots that was done to verify the presence of MON 89788 in MON87705xMON89788 (*Section A2.2.2 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.

  OBS.: The applicant does not show any southern blot analysis for Generational Stability.
  They have done it for the MON 87705, but is does not mean that the stability will be the same.
  And the southern blot picture for this analysis has a bad quality **(**Skipwith et al, 2009 – p.59**)**.
  The ~5.7 kb band is the expected size for the border fragment containing the 3' end of the inserted DNA (T-DNA I and T-DNA II) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure 3). However, the migration of this fragment appears slightly lower than indicated by the molecular weight marker most likely due to differences in salt concentrations between the samples and marker (Skipwith et al, 2009 – p.30).

<u>Comments and recommendations on organization and sequence of the inserted genetic material at each insertion site *(Section A2.2.2 ii)*</u>

MON87705

- All the information about organization and sequence of this new GM are the same as the one used the Application EFSA-GMO-NL-2010-78 Monsanto Company (2010).
- The applicant claims that **(p.33)** "*Since the inserts present in MON 87705 × 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 87705 identified a 36 bp deletion of soybean genomic DNA sequence and a 2374 bp insertion just 5' to the MON 87705 insertion site. Also, there are 4 new bases located at the 3' junction of the insert. On this duplicated 2374bp, there is a single nucleotide change (A → T).
- "*Given the very high homology between the 2374 bases flanking the 5' end of the insert and the genomic DNA flanking the 3' end of the insert, the 2374 bases are most likely from the 3' end of the flanking genomic DNA of the insertion site and were duplicated at the 5' end of the insertion site when T-DNA I and T-DNA II integrated into the genome.*" **(p.72 Application MON87705 – 2010).** What is the function of this 2374bp sequence? Could it be part of a gene? If it is part of a gene, could it be over-expressing some characteristic? The applicant should provide the mRNAs from this duplicated sequence. The applicant should provide a better picture of the PCR gel in this study.
- *As expected, a PCR product across the insert in MON 87705 was not generated in this analysis since the PCR conditions to generate a product of this size (13,349 bp) were not used* (Skipwith, 2009 - p.28). A better enzyme could have been used to amplify this fragment.
- The Applicant do 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 a independent laboratory should be used.

MON89788

- The applicant should provide a better picture of the PCR gel in this study.
- 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 a independent laboratory should be used

Assessment of the newly expressed protein *(Section 2.2.3)*

In the dossier the Applicant look at the expression patterns of glyphosate treated MON87705xMON89788, and comparing it to two positive control plants (MON 87705 and MON89788) in different fields.

The level of expression of Cp4EPSPS protein is analyzed in forage and seed and is analyzed at different growth stages representative for these types of tissues. Other parts of the plant are not considered as relevant. Protein extract from a conventional soy tissue is used as negative control.

The level of Cp4-EPSPS is found to be additive in forage, which can be expected in a combined expression for MON87705 and MON89788, but not in seed (**Table 4, Application for Authorization / dossier, p.37**). This difference is not discussed further. However, the level of protein is considered as low and not considered to cause any harm when used in food/feed.

The level of FAD2-1A/FATB1-A is not considered relevant for this section by the applicant as it is not a protein but a double stranded RNA not considered interacting with the Cp4-EPSPS protein. The applicant also states that there is no known mechanism for the interaction between various mRNA and/or protein products of MON87705 and MON87798 that could cause harm to humans/animals. However, there is no reference to this statement others than the high degree of substrate specificity that they have. The substrate specificity of EPSPS is analyzed in **EFSA-GMO-NL-2010-78, Monsanto, p214, section D.7.8.1.**

Recommendation: The assertion *" that there is no known mechanism for the interaction between various mRNA and/or protein products of MON87705 and MON87798 that could cause harm to humans/animals"* should be supported by scientific data and/or references

Toxicity and allergenicity assessment *(Section 4 and 5)*

Assessment for toxicity of the newly expressed proteins are based on the criteria of: history of safe use, no structural similarity to known toxins, no acute toxicity to mammals, low concentration in consumed tissues and rapid digestibility in simulated digestive fluids. EFSA (EFSA 2008) has previously expressed a positive opinion of the safety of introduced Cp4-EPSPS protein in MON89788 and this single event has been approved for food, feed, and import/processing in 2008.

From the data provided, our understanding is that the studies performed on safety of the expressed protein Cp4-EPSPS is not performed on the plant version, but on the recombinant *E.coli* version of the protein. From our point of view, the plant version should be used for such purposes even though the concept of equivalence is proven by structure analysis (sequencing). Plants and bacteria do differ in their post-translational processing of proteins, and this is not considered.

The heating of the protein affects the proteins relative activity from 37°C and higher. At the highest temperature used (95°C), less than 8 % activity is found. Thus, the activity is not completely lost, indicating that the heat treatment performed is not sufficient for a complete denaturation of the EPSPS

protein from *E.coli*. The plant version of the protein is not tested, and it is therefore not possible to draw any conclusion on that. The heat treatment of EPSPS for both 15 and 30 minutes also do not seem to affect the appearance of the protein on the gel after SDS-PAGE. The protein seems to be quite stable. The pH analysis (influences of pH on the purified protein) indicates the same.
The glycosylation analysis of Cp4-EPSPS refers to analysis of the proteins expressed in MON87705 and MON87988 separately, and not compared to the Cp4-EPSPS expressed in the combined new event MON87705 X MON87798.
No repeated rodent 28 day oral dose toxicity study or a 90-day toxicity study is performed with the plant version of the protein isolated from MON87705xMON87798 because of the "evidenced" safety of the protein from *E.coli*.
Thus, there is also no data on FAD2-1A/FATB1-A alone on toxicity in MON87705x MON87798.
Regarding allergy, no allergic potential has been found in the assessment of MON87705 and MON87798 separately (source, structural similarity to known allergens, digestibility in simulated gastric fluids, serological studies and low proportion of total protein). The combined event MON87705xMON87798 and a potential of increased allergenicity is discussed and found not relevant. This potential is not investigated further as the applicant claims that the allergenic potential is inherited from the single events. There is however no referenced data on this. Other than the fact that soy is considered as an allergenic food itself (Burks et al 1988).

Recommendation: Use plant version of the protein(s)
Perform analysis on the combined event (MON87705xMON87798) and base conclusions on that rather than on the single events separately.

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

Social utility and sustainability aspects

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

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

These issues are further detailed in the regulation on consequence assessment section 17 and its annex 4. The Applicant has not provided relevant information that allows an evaluation of the issues laid down in the aim of the Act, regarding ethical values, social justification of the GMO within a sustainable development. Given this lack of necessary information for such an evaluation, the Applicant has not demonstrated a benefit to the community and a contribution to sustainable development from the use of MON87705xMON89788. 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 MON87705xMON89788 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 MON87705xMON89788 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 MON87705xMON89788. 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 MON87705xMON89788 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.

References

Application EFSA-GMO-NL-2006-36 Monsanto Company (2006).

Application EFSA-GMO-NL-2010-78 Monsanto Company (2010).

Axelrad JC, Howard CV, Mclean WG, (2003). The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon. Toxicology 185:67-78.

Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S, Plaetinck G, Munyikwa T, Pleau, M (2007). Control of coleopteran insect pests through RNA interference. Nat Biotechnol *25*, 1322-1326.

BAT. Biosafety Assessment Tool (GenØk and University of Canterbury). www.bat.genok.org/bat.

Benbrook CM, 2003. Impacts of Genetically Engineered Crops on Pesticide Use in the United States: The First Eight Years. pp. 1-42.

Blackburn LG, Boutin C, (2003). Subtle effects of herbicide use in the context of genetically modified crops: A case study with glyphosate (Roundup (R)). Ecotoxicology 12:271-285.

Burks AW, Jr., Brooks JR and Sampson HA (1988). Allergenicity of major component proteins of soybean determined by enzyme-linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. Journal of Allergy and Clinical Immunology, 81, 1135-1142.

CBD (2003). Cartagena Protocol on Biosafety. http://www.cbd.int/biosafety/

CENTER FOR ENVIRONMENTAL RISK ASSESSMENT (CERA) (2011). Problem Formulation for the Environmental Risk Assessment of RNAi Plants. Conference proceedings document, June.

Codex, 2003. Principles For The Risk Analysis Of Foods Derived From Modern Biotechnology; Codex Alimentarius Commission, CAC/GL 44-2003

Codex (2003a). Codex Work on Foods Derived from Biotechnology. In CAC/GL 45-2003. Codex. http://www.who.int/foodsafety/biotech/codex_taskforce/en/.

Cuhra M, Traavik T and Bøhn T (2012) Colone- and age-dependent toxicity of a glyphosate commercial formulation and ist active ingredient in Daphnia magna. Ecotoxicology, DOI 10.1007/s10646-012-1021-1

Dallegrave E, Mantese FD, Coelho RS, Pereira JD, Dalsenter PR, Langeloh A (2003). The teratogenic potential of the herbicide glyphosate-Roundup (R) in Wistar rats. Toxicology Letters 142:45-52.

De Schrijver A, Devos Y, Van den Blucke M, Cadot P, De Loose M, Reheul D and Sneyer M (2006) Risk assessment of GM stacked events obtained from crosses between GM Events. Trends in Food and Sci Technol XX, 1-9.ONDde69

Dill GM, Sammons RD, Feng PCC, Kohn F, Kretzmer K, Mehrsheikh A, Bleeke M, Honegger JL, Farmer D, Wright D, Haupfear EA (2010) Glyphosate: discovery, development, applications, and

properties. In: Nandula VK (ed) Glyphosate resistance in crops and weeds: history, development, and management.Wiley, New York, pp 1–33

Dolezel M, Miklau M, Eckerstorfer M, Hilbeck A, Heissenberger A, Gaugitsch H, (2009) Standardising the Environmental Risk Assessment of Genetically Modified Plants in the EU / Standardisierung der Umweltrisikoabschätzung gentechnisch veränderter Pflanzen in der EU. BfN – pp. 259.

EC, Regulation 1829/2003.

EFSA 2008: EFSA-GMO-NL-2006-36, EFSA journal (2008)758:1-23

Gordon KHJ and Waterhouse PM (2007). RNAi for insect-proof plants. Nat Biotechnol *25*, 1231-1232.

Halpin C (2005) Gene stacking in transgenic plants- the challenge for 21st centry plant biotechnology. Plant Biotechnol, 3:141-155.

Heinemann JA (2009). Hope not Hype. The future of agriculture guided by the International Assessment of Agricultural Knowledge, Science and Technology for Development (Penang, Third World Network).

Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P, (2003). Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (Oreochromis niloticus). Environmental Toxicology 18:260-267.

Mao Y-B, Cai W-J, Wang J-W, Hong G-J, Tao X-Y, Wang L-J, Huang Y-P and Chen X-Y (2007). Silencing a cotton bollworm P450 monooxygenase gene by plant mediated RNAi impairs larval tolerance of gossypol. Nat. Biotechnol. *25*, 1307-1313.

Marc J, Mulner-Lorillon O, Boulben S, Hureau D, Durand G, Belle R (2002). Pesticide roundup provokes cell division dysfunction at the level of CDK1/cyclin B activation. Chemical Research in Toxicology 15:326-331.

Mesnage R, Clair E, Gress S, Then C, Szekacs A and Seralini G-E (2012) Cytotoxicity on human cells of Cry1AB and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. Appl Toxical, doi: 10.1002/jat.2712.

Ono, MA, Itano EN, Mizuno LT, Mizuno EHF, Camargo ZP (2002). Inhibition of Paracoccidioides brasiliensis by pesticides: Is this a partial explanation for the difficulty in isolating this fungus from the soil? Medical Mycology 40:493-499.

Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE (2005). Differential effects of glyphosate and roundup on human placental cells and aromatase. Environmental Health Perspectives 113:716-720.

Séralini GE, Clair E, Mesnage R, Gress S, Defarge N, Malatesta M, Hennequin D, de Vendômois JS (2012) Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food Chem Toxicol. 2012 Nov;50(11):4221-31. doi: 10.1016/j.fct.2012.08.005.

Skipwith A, Lawry KR, Tian Q and Masucci JD (2009) Amended Report for MSL0022130: Molecular Analysis of Soybean MON 87705. Monsanto Company

Solomon KR, Thompson DG (2003). Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. Journal of Toxicology and Environmental Health-Part B Critical Reviews 6:289-324.

Then C (2009) Risk assessment of toxins derived from Bacillus thuringiensis – synergism, efficacy, and selectivity. Environ Sci Pollut Res DOI 10.1007/s11356-009-0208-3.

Zhang et al. (2012) Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Research v.22, p.107-126.