Vår ref: 2015/H_125
Deres ref: 2015/11755

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 22.12.15

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på offentlig høring av søknad **EFSA/GMO/BE/2015/125**, mais event MON87403, fra Monsanto Company under EU forordning 1829/2003. Søknaden gjelder mat, fòr, import og prosessering av genmodifisert mais **MON87403.**

Vennligst ta kontakt hvis det er noen spørsmål.

Med vennlig hilsen,

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Vår ref: 2015/H_125
Deres ref: 2015/11755

Assessment of the summary of the technical dossier of EFSA/GMO/BE/125 maize event MON87403 under EC regulation1829/2003.

Sent to

Norwegian Environment Agency

by

GenØk- Centre for Biosafety
December 2015

KONKLUSJON PÅ NORSK

Hovedkonklusjon og anbefalinger:
Genøk–Senter for Biosikkerhet viser til brev fra Miljødirektoratet angående offentlig høring i EU for **MON87403 mais** i bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **MON87403 mais.**

Ut ifra vurderingskriteriene for bærekraft, samfunnsnytte og etiske aspekter, gir ikke søker opplysninger som belyser disse i henhold til det som forutsettes anvendt i den norske genteknologilovens (Appendix 4).

I denne sammenheng er det viktig å få dokumentert erfaringer med hensyn på effekter på miljø, helse og samfunnsaspekter. Denne type dokumentasjon er ikke tilstrekkelig i den oppsummerte søknaden om omsetting av **MON87403 mais** til import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **MON87403 mais.**

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av **MON87403 mais** til import og prosessering og til bruk i fòr og mat basert på dette.

Vår ref: 2015/H_125
Deres ref: 2015/11755

Assessment of the summary of the technical dossier of EFSA/GMO/BE/125 maize event MON87403 under EC regulation1829/2003.

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 assessment of product safety and corresponding impact assessment of event **MON87403 maize**, setting out the risk of adverse effects on the environment and health, including other consequences of proposed release under the pertinent Norwegian regulations.

Specific recommendations

Based on our findings, we propose some specific recommendations, summarized here and detailed in the go-through below.

- We encourage the Applicant to further investigate the 149bp deletion in the maize genome and what potential effects it might have on other genes and their expression.
- We encourage the applicant to verify if the 35S promoter used, contain ORFs and if there are any phenotypic changes resulting from that (as in unintended protein expression).
- We encourage the Applicant to specify the source of proteins used for safety analysis, also in the summary of the technical dossiers.
- We encourage the Applicant to clarify whether or not glycosylation analysis were performed and if functional analysis of the protein was performed.
- We emphasize the importance of environmental monitoring plans when it comes to introduction of new genetic traits into the environment.
- MON87403 is modified for increased ear biomass during early reproduction and this is expected to result in increased grain yield. When assessing sustainability it is important to consider whether optimal performance of the MON87403 maize depends on increased use of other input factors such as water and fertilizers, and whether this will increase farmers' production costs.
- It is important to consider whether MON87403 may be more attractive to pests and pathogens due to the increased ear biomass, and strategies used to control these pests/pathogens.
- It is important to consider whether the grains have different storage qualities or nutritional qualities as a food and feed source.
- The MON87403 maize has not yet been approved for cultivation in a third country. It cannot be expected that the performance of MON87403 will be the same and that the same effects will apply between different social and environmental contexts (Fisher et al., 2015). A proper evaluation of potential social impacts of relevance to sustainability can therefore not be completed until this event has been approved for cultivation in a third country, so that information relevant for the socio economic impacts assessment in the producing countries can be provided. This includes information about the share of the benefits among sectors of the society, and particularly whether cultivation of MON87403 changes farmers' opportunity to reuse and share seeds.
- The cultivation of GM plants in general is causing problems with regard to co-existence. An evaluation of the occurrence of volunteer plants in the producing countries, suggested control strategies, as well as information about the strategies adopted to ensure co-existence with conventional and organic maize production and potential consequences for these production forms in the producing countries is required for a socio economic and sustainability assessment.
- It is also important to evaluate whether alternative options (e.g. the parental non-GM version of this MON87403 maize) may achieve the same outcomes in a safer and ethically justified way. Furthermore, in order to evaluate whether The MON87403 maize contributes to social utility, it is important to consider current and future demand

for this GM-maize product for food, feed and processing purposes in Norway and to what extent this demand is/can be satisfied by existing sources.

Overall recommendation

In our assessment of maize event MON87403, we find that the information provided in the summary of the technical dossier does not provide enough data to support claims of safe use, social utility and sustainable development.

We therefore conclude that the Applicant has not provided the information required under Norwegian law to warrant approval in Norway at this time.

Especially, the Applicant has not included information which is required to assess social utility and sustainability as required by the Norwegian Gene Technology Act (Appendix 4) for consideration of approval in Norway.

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.

ASSESSMENT OF THE SUMMARY OF THE TECHNICAL DOSSIER OF EFSA/GMO/BE/2015/125MAIZE UNDER EU REGULATION 1829/2003.

About the event

Maize event MON87403 was made through *Agrobacterium tumefaciens* mediated transformation of maize tissue with the vector PV-ZMAP5714 containing a gene from *Arabidopsis thaliana*, called **ATHB17**. This gene encodes a protein that modulate plant growth and development and also regulates gene expression. The resulting maize plant has an increased (ear) biomass.

Maize event MON87403 is not approved for any applications in Norway or EU.
Applications for approval for all applications has been sent to Canada, Japan, Korea and US. Applications are also planned to be sent to countries importing high amounts of maize to food and feed and that have a regulatory approval system.

Maize event MON87403 is not approved for any applications in a third country.

ASSESSMENT FINDINGS

The assessment finding are based on the summary of the technical dossier, the safety assessment report from Food standards Australia New Zealand (FSANZ) and other per reviewed data, if available.

Molecular characterization of ATHB17.
The MON87403 maize has been genetically modified through *A. Tumefaciens* mediated transformation with the ATHB17 expression cassette (application). This cassette contains the full-length gene for the ATHB17 protein, which is involved in regulating gene expression in the plant through dimerization and binding to DNA (Rice et al. 2014), and thereby functions as a transcriptional repressor. However, maize-specific splicing of the ATHB17 transcript results in a truncated version of the protein, ATHB17Δ113. This truncated version of the protein remains its ability to dimerize but lacks a functional repression domain, and works in a dominant-negative manner to modulate HD-Zip II-regulated pathways. Rice et al. (2014) demonstrated that the HD-Zip II protein functions as a transcriptional supressor, and that the expression of the ATHB17Δ113 in maize leads to increased ear silking and thus maize biomass (Rice et al. 2014). The applicant also shows that the MON87403 expressing this truncated protein led to improved sink tissue when compared to control plants.

Insert number and presence of backbone
Next generation sequencing (NGS), Junction Sequence Analysis (JSA) and PCR was used to investigate the number of the insertion sites of the T-DNA, its flanking region and to confirm that no plasmid backbone was present in the MON87403 genome. In the transformation process, herbicide tolerance and antibiotic tolerance were used as selection markers, and was not intended to be transferred to the plant. The NGS indicated that the T-DNA was inserted at one single location, and that no backbone was present. This was further confirmed by junction sequence analysis that identified two unique junction sequences indicating one single DNA-insertion in the MON87403. A non-transformed plant was used as negative control. In addition, PCR was run to further confirm the presence, the sequence of the insertion site and confirm the absence of the plasmid backbone (FSANZ Supporting Document 1).

Insertion site analysis
Two specific primers to the 5' and 3' flanking end were used to map the insertion site by PCR of genomic DNA isolated from the control plant (LH244). This product was sequenced and compared to that obtained from the 5' and 3' region of the ATHB17 expression cassette (FSANZ Supporting Document 1). This demonstrated a deletion of 149 bp that had occurred during the transformation (application). The applicant does not further investigate or comment on the nature of this deleted region.

Genetic and phenotypic stability
The genetic stability was investigated through analysis of NGS/JSA from grains of five generations, confirming the presence of the ATHB17 expression cassette (FSANZ Supporting Document 1). Phenotypic stability was analyzed through Chi-square (x^2) analysis over several generations based on the expectations of a Mendelian inheritance pattern due to the single insertion site. This investigation supported the conclusion of a single insertion site in the MON87403 genome (FSANZ Supporting Document 1).

Conclusion
The transformation event of the MON87403 has been thoroughly investigated through several molecular analysis. All analysis performed support that the ATHB17 expression cassette has a single insertion site in the plants genome, and that there are no backbone plasmid present. The MON87403 therefore thus not contain the genes for herbicide tolerance nor antibiotic resistance.

The applicant does not comment on the 149 bp deletion in the maize genome, which happened upon transformation. The applicant has the tools and the knowledge to map this section and investigate whether the insert disturbs a gene in the maize genome through mapping of open reading frames and bioinformatics analysis. Thereby the applicant can comment on what this deletion might imply to the maize plant.

Recommendation

- We encourage the Applicant to further investigate the 149bp deletion in the maize genome and what potential effects it might have on other genes and their expression.

The e35S promoter
Safety questions related to the use of the Cauliflower Mosaic Virus 35S promoter (P35S) in GM plants has recently been discussed in an article from Podevin and Du Jardin (2012). In the article, the authors state that some P35S variants contain open reading frames that when expressed could lead to "unintended phenotypic changes. Gene VI encodes the multifunctional P6 protein that can be divided into four domains (Li and Leiser, 2002). Functions of P6 include nuclear targeting (Haas et al. 2008), viral particle binding and assembly (Himmelbach et al. 1996), si- and ds-RNA interference and interference suppression (Shivaprasad et al. 2008) and transcriptional transactivation (Kobayashi et al 2004, Palanichelvam et al. 2002).

Recommendation:

- We encourage the applicant to verify if the 35S promoter used, contain ORFs and if there are are any phenotypics changes resulting from that (as in unintended protein expression).

Information on the expression of the insert.
According to the summary of the technical dossier, the protein levels of ATHB17Δ113 were determined by enzyme-linked immunosorbent assay (ELISA) in tissues. Data from these analysis are not available for us. Thus, we cannot comment on levels in different tissues and especially not for the tissues considered as the most relevant ones, grain and forage.

Toxicology and allergenicity
According to the summary of the technical dossier, the ATHB17Δ113 protein is the only novel protein expressed in maize event MON87403. The safety assessments are therefore focusing on ATHB17Δ113 only.

The toxicity assessment is based on the following characteristics and comparisons

- History of safe use of the protein.
- Structural similarity to known toxins or other biologically active proteins causing adverse effects.
- How rapid the protein is digested in mammalian gastrointestinal systems.

It is not clear from the summary of the technical dossier if it is the plant or the bacterial version of the ATHBΔ113 protein that is used for the safety assessment.

However, the Safety Assessment Report provided by FSANZ (Supporting document 1) claims that it is an *E.coli* produced protein that is used for the safety evaluations sent to their countries. This is done because the protein produced by the plant is "insufficient for safety analysis".
It is most likely the case for the application sent to EU as well, although not clearly stated.

The applicant claim that the protein ATHB17Δ113 is not hazardous to humans based on the fact that the donor organism protein (ATHB17) does not pose any harm to humans, animals or environment. There are also not no sequence similarities to known toxins and the protein is rapidly degraded in gastric systems.
We do however have no experimental data supporting these claims. It is also not clear if analysis of glycosylation of the protein was performed or to what degree functional analysis was performed of the plant derived MON87403 protein.

The allergenicity assessment is based on the following criteria:

- Source of protein is allergenic or not
- Structural similarity to known allergens
- How rapid the protein is digested in an in vitro assay of the mammalian gastrointestinal system

Based on these criteria the proteins is considered non-allergenic. We do however not have access to the full dossier, and cannot comment of results from studies performed to support the statement by the applicant.

Recommendation:

- We encourage the Applicant to specify the source of proteins used for safety analysis, also in the summary of the technical dossiers.
- We encourage the Applicant to clarify whether or not glycosylation analysis were performed and if functional analysis of the protein was performed.

Environmental risk assessment (ERA) and monitoring plan

We emphasize the crucial role of the agricultural context in which these crops will be grown. There are several risks connected to the cultivation of genetically modified crops, among them gene flow (both to non-modified crops and wild relatives of the crop) and potential impacts on the surrounding ecosystems through affecting insect and plant life, small mammals and birds and aquatic life (i.e. non-target organisms) (Warwick et al. 2009).

Recommendation:

- We emphasize the importance of environmental monitoring plans when it comes to introduction of new genetic traits into the environment.

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 (NGTA). In accordance with the aim of the NGTA, 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 elaborated in the regulations relating to impact assessment pursuant to the NGTA, section 17 and its annex 4. The NGTA, with its clauses on societal utility and sustainable development, comes into play with a view also to health, environmental and socio-economic impacts in other countries, such as where the GMOs are grown.

The Applicant has not provided information relevant for the assessment of the social utility of the MON87403 maize and its contribution to sustainable development. We suggest some issues that we consider relevant to evaluate in order to assess these criteria, and encourage the regulator to ask the Applicant to submit relevant information.

Recommendations:

- MON 87403 is modified for increased ear biomass during early reproduction and this is expected to result in increased grain yield. When assessing sustainability it is important to consider whether optimal performance of the MON87403 maize depends on increased use of other input factors such as water and fertilizers, and whether this will increase farmers' production costs.

- It is important to consider whether MON 87403 may be more attractive to pests and pathogens due to the increased ear biomass, and strategies used to control these pests/pathogens.
- It is important to consider whether the grains have different storage qualities or nutritional qualities as a food and feed source.
- The MON 87403 maize has not yet been approved for cultivation in a third country. It cannot be expected that the performance of MON 87403 will be the same and that the same effects will apply between different social and environmental contexts (Fisher et al., 2015). A proper evaluation of potential social impacts of relevance to sustainability can therefore not be completed until this event has been approved for cultivation in a third country, so that information relevant for the socio economic impacts assessment in the producing countries can be provided. This includes information about the share of the benefits among sectors of the society, and particularly whether cultivation of MON 87403 changes farmers' opportunity to reuse and share seeds.
- The cultivation of GM plants in general is causing problems with regard to co-existence. An evaluation of the occurrence of volunteer plants in the producing countries, suggested control strategies, as well as information about the strategies adopted to ensure co-existence with conventional and organic maize production and potential consequences for these production forms in the producing countries is required for a socio economic and sustainability assessment.
- It is also important to evaluate whether alternative options (e.g. the parental non-GM version of this MON 87403 maize) may achieve the same outcomes in a safer and ethically justified way. Furthermore, in order to evaluate whether The MON 87403 maize contributes to social utility, it is important to consider current and future demand for this GM-maize product for food, feed and processing purposes in Norway and to what extent this demand is/can be satisfied by existing sources.

Conclusion

The Applicant does not attempt to identify socio-economic implications, nor demonstrate a benefit to the community and a contribution to sustainable development from the use of the MON 87403 maize and does therefore not provide sufficient information as required by the NGTA

References:

Food Standards Australia New Zealand (FSANZ) Supporting Document 1: Safety report-Application A1114. Food derived from high yield corn line MON87403

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