Vår ref: 2016/H_127
Deres ref: 2016/2722

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 18.04.16

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på offentlig høring av søknad **EFSA/GMO/NL/2015/127**, genmodifisert mais 1507 x MIR162 x MON810 x NK603, fra Pioneer Hi-Bred International, Inc., under EU forordning 1829/2003. Søknaden gjelder bruksområdene mat, fòr, import og prosessering.

Vennligst ta kontakt hvis det er noen spørsmål.

Med vennlig hilsen,

Idun Merete Grønsberg
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Vår ref: 2016/H_127
Deres ref: 2016/2722

Assessment of the summary of the technical dossier of EFSA/GMO/NL/2015/127 maize event 1507 x MIR162 x MON810 x NK603 under EC regulation1829/2003.

Sent to

Norwegian Environment Agency

by

GenØk- Centre for Biosafety
April 2016

KONKLUSJON PÅ NORSK

Hovedkonklusjon og anbefalinger:
Genøk–Senter for Biosikkerhet viser til brev fra Miljødirektoratet angående offentlig høring i EU for **genmodifisert mais 1507 x MIR162 x MON810 x NK603** i bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **1507 x MIR162 x MON810 x NK603** mais.

Vår anbefaling er at norske myndigheter ikke godkjenner bruk av **1507 x MIR162 x MON810 x NK603 mais** til import og prosessering og til bruk i fòr og mat basert på dette.

Dette begrunnes utifra vurderingskriteriene for bærekraft, samfunnsnytte og etiske aspekter, der søker ikke gir 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 **1507 x MIR162 x MON810 x NK603 mais** til import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **1507 x MIR162 x MON810 x NK603 mais.**

Vår ref: 2016/H_127
Deres ref: 2016/2722

Assessment of the summary of the technical dossier of EFSA/GMO/NL/2015/127 maize event 1507 x MIR162 x MON810 x NK603 under EC regulation1829/2003.

As a designated National Competence Center for Biosafety, GenØk aims to provide advice giving which is independent and holistic and with a 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 1507 x MIR162 x MON810 x NK603 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.

- Based on the information in the summary of the present Application for 1507 x MIR162 x MON810 x NK603 we encourage the Applicant to use more than one detection method for this region of the construct to verify presence of 1507 insert.
- Based on the lack of information in the summary of the present Application for 1507 x MIR162 x MON810 x NK603 we encourage the Applicant to investigate further upon the potential for allergenic reactions of Cry toxins, especially since this multistack contains several of these family of proteins, together with the potential for adjuvance effects of these.
- Based on the information in the summary of the present Application for 1507 x MIR162 x MON810 x NK603 we encourage the Applicant to provide data showing that there is no risk of increased development of resistance in target or non-target organisms.
- Based on the information in the summary of the present Application for 1507 x MIR162 x MON810 x NK603 we question the acceptance of use of the herbicide glufosinate-ammonium that would impose serious health risks for workers.
- The Applicant should demonstrate the lack of interactive effects between transgenic proteins in this **stacked event** through proper scientific testing and evidence gathering, rather than justify the lack of testing based on assumptions-based reasoning of no effects.
- The regulator is encouraged to address the potential of non-target effects of **Bt toxins.**
- The regulator is encouraged to consider the safety of co-products (herbicides used) intended to be used with the GM event in the evaluation of safety.
- The combined effect of potential allergens in the stack should be investigated.
- 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.
- Applicant should provide evidence of a lack of toxicological effects from interactions of the newly expressed transgenic proteins in the event under consideration in relation to their singular events.
- The potential for cross resistance between Cry- and VIP proteins and a changed effect on target and also non-target species should be investigated.
- The potential for non-target effects of VIP proteins should be investigated, and should include species not of the order Lepidoptera.
- The Applicant should survey for Vip3A resistance alleles prior to the use of this toxin.
- The applicant should include a full evaluation of the co-technology intended to be used with 1507 x MIR162 x MON810 x NK603, namely glyphosate- and glufosinate ammonium based herbicides. Particular focus should be given to the level of accumulation of herbicides in the plants, particularly the parts used in food and feed production, and whether or not these levels of exposure could cause acute and/or chronic health issues.

- The Applicant is encouraged to characterize the distinct inserts as they are present in the multistack of this Application, and not based on previous characterizations of the single events.
- 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).
- We encourage the Applicant to specify the source of proteins used for safety analysis, also in the summary of the technical dossiers.
- We also encourage the Applicant to consider the combined expression of the distinct proteins in the whole plant in the analysis of toxicology and allergy.
- We emphasize the importance of environmental monitoring plans when it comes to introduction of new genetic traits into the environment.
- In order to meet the requirements for the NGTA, the regulator is encouraged to ask the Applicant to submit information relevant for the assessment of the social utility of the 1507 x MIR162 x MON810 x NK603 maize and its contribution to sustainable development. The information provided by the Applicant must be relevant for the agricultural context in the producing country/countries. The information should include issues such as: development of pest resistance in target populations, impacts on non-target organisms, herbicide resistance in weed populations, co-existence consequences and possible impacts among poor and/or small-scale farmers in producing countries and share of the benefits among sectors of the society.

Overall recommendation

In our assessment of maize event 1507 x MIR162 x MON810 x NK603, 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 comment 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/NL/2015/127, 1507 x MIR162 x MON810 x NK603 MAIZE UNDER EU REGULATION 1829/2003.

About the event

Maize event 1507 x MIR162 x MON810 x NK603 is a stacked event variety expressing three *Bacillus thuringiensis* (Bt) proteins to confer resistance to lepidopteran insects (Cry1Ab from MON810, Cry1F from 1507 and vip3Aa19 from MIR162). It also expresses two proteins that confer tolerance to the herbicides glufosinate ammonium (PAT from 1507) and glyphosate (CP4 EPSPS from NK603).
In addition, a selection marker is expressed (PMI used during establishment of parental line MIR162).

Maize event 1507 x MIR162 x MON810 x NK603 is not approved for any application in Norway or EU. However, approval is given for the parental lines in EU.

Applications for approval has been sent to US, Canada, Brazil, Columbia and Argentina.

Applications will also be sent to countries with regulatory approval systems for approval of stacked transgenic products.

We have previously assessed the following combinations of the transgenic events in this stack:

- MON89034 x 1507 x NK603 (EFSA/GMO/NL/2009/65), in 2010.
- MON89034 x NK603 (EFSA/GMO/NL/2009/72), in 2010.
- BT11 x MIR162 x 1507 x GA21 (EFSA/GMO/DE/2010/86) in 2012.
- 1507 x 59122 x MON810 x NK603 (EFSA/GMO/NL/2011/92) in 2012.
- MON810 pollen (EFSA/GMO/NL/2012/107), in 2012.
- BT11 x 59122 x MIR604 x 1507 x GA21 (EFSA/GMO/BE/2011/99), in 2012.
- BT11 x MIR162 x MIR604 x 1507 x 5307 x GA21 (EFSA/GMO/DE/2011/103), in 2014.
- MON87427 x MON89034 x NK603 (EFSA/GMO/BE/2013/117), in 2015.
- MON87427 x MON89034 x 1507 x MON88017 x MON59122 (EFSA/GMO/2013/118), in 2015.

ASSESSMENT FINDINGS

The assessment finding are based on the summary of the technical dossier, previous assessments of combinations of this multistack and other per reviewed data, if available.
In this section, recommendations and comments made to the relevant inserts for the multistack in this application is noted.

TC1507: Single nucleotide polymorphism (SNP) has been detected in the promoter region (Morriset et al. 2009). This SNP negatively effects detection of this event by some methods (ENGL used methods).

Recommendation:

- Based on the information in the summary of the present Application for 1507 x MIR162 x MON810 x NK603 we encourage the Applicant to use more than one detection method for this region of the construct to verify presence of 1507 insert.

In investigations with Cry1Ab protein, Guimaraes et al. (2008) did not find a similar type of adjuvant effect elicited against peanut proteins as with Cry1Ac, yet instead found evidence of Cry1Ab acting as an adjuvant leading to early phase production of leukotrienes and increased Th2 and Th17- cytokine production in branchoalveolar lavage fluids after airway exposure. The implication of possible effects of Cry1Ab to produce allergen-induced cytokine responses is an area of investigation warranting further inquiry.

Recommendation:

- Based on the lack of information in the summary of the present Application for 1507 x MIR162 x MON810 x NK603 we encourage the Applicant to investigate further upon the potential for allergenic reactions of Cry toxins, especially since this multistack contains several of these family of proteins, together with the potential for adjuvance effects of these.

Tabashnik et al (2009) found evidence of reactivity among "pyramided" (stacked events) of Cry1Ac and Cry2B endotoxins in transgenic cotton. The cross reactivity led to higher rates of resistance evolution in pink bollworm, Pectinophora gossypiella, in a laboratory setting. Their results suggest that in the case of different Cry protein species, cross reactivity between them may confer increased rates of insect resistance that would alter the efficacy and perhaps biological activity of the GMO.

Recommendation:

- Based on the information in the summary of the present Application for 1507 x MIR162 x MON810 x NK603 we encourage the Applicant to provide data showing that there is no risk of increased development of resistance in target or non-target organisms.

Glufosinat ammonium is not legal for use in Norway and in EU (except a limited use on apples) due to both acute and chronic effects on mammals including humans.
According to EFSA (EFSA Journal 2015), *the use of glufosinate ammonium will lead to exposures that exceed acceptable exposure levels during application. Accordingly, more strict*

laws have been introduced in EU from 2007. If 1507 x MIR162 x MON810 x NK603 is grown outside EU, but imported and used within, one would have to accept that farmers in countries with less strict regulations are under significant health risks.

Recommendation:

- Based on the information in the summary of the present Application for 1507 x MIR162 x MON810 x NK603 we question the acceptance of use of the herbicide glufosinate ammonium that would impose serious health risks for workers.
- The Applicant should demonstrate the lack of interactive effects between transgenic proteins in this **stacked event** through proper scientific testing and evidence gathering, rather than justify the lack of testing based on assumptions-based reasoning of no effects.
- The regulator is encouraged to address the potential of non-target effects of **Bt toxins.**
- The regulator is encouraged to consider the safety of co-products (herbicides used) intended to be used with the GM event in the evaluation of safety.
- The combined effect of potential allergens in the stack should be investigated.

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, de Schrijver et al, 2007). 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.

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.

According to the applicant EFSA has no safety concerns regarding any interactions between the two enzymes C4EPSPS and PAT as they have been evaluated in previous stacks that include the transgenic proteins in question (EFSA 2008, EFSA 2009).
The interaction between the different Cry proteins has yet not been specifically assessed by EFSA.

Recommendation:

- Applicant should provide evidence of a lack of toxicological effects from interactions of the newly expressed transgenic proteins in the event under consideration in relation to their singular events.

Stacked events

A stacked event has to be regarded as a new event, even if no new modifications have been introduced. The gene-cassette combination is new and only minor conclusions could be drawn from the assessment of the parental lines, since unexpected effects (e.g. synergistic effects of the newly introduced proteins) cannot automatically be excluded.

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, de Schrijver et al, 2006).

The 1507 x MIR162 x MON810 x NK603 maize combines several classes of Bt proteins active against insects pest like Lepidoptera. It is well known that synergistic and additive effects both between Bt toxins and other compounds do occur (Then, 2009). 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 and must be carefully considered in the development and risk assessments of stacked events. Robust data are necessary to identify whether the combined presence of transgenes influences expression levels.

Safety of Cry genes

As already mentioned, 1507 x MIR162 x MON810 x NK603 maize combines different classes of Bt proteins named Cry toxins (Cry1Ab, Cry1F) and vip3Aa19 (Vip proteins is dicussed in a separate chapter, p11). These toxins are claimed and believed to be safe, however lately the potential of non-target effects of Bt toxins concerning mode of action have been addressed (Gilliand et al 2002, Crickmore 2005, Hilbeck and Schmidt 2006). A review by (Hilbeck and Schmidt 2006) on all Bt-plants found 50% of studies documenting negative effects on tested invertebrates.

In relation to non-target and environmental effects, in two meta-analyses of published studies on non-target effects of Bt proteins in insects, (Lövei and Arpaia 2005) documented that 30% of studies on predators and 57% of studies on parasitoids display negative effects to Cry1Ab transgenic insecticidal proteins. A review by (Hilbeck and Schmidt 2006) on all Bt-plants found 50% of studies documenting negative effects on tested invertebrates.
Another quantitative review by (Marvier et al. 2007) suggested a reduction in non-target biodiversity in some classes of invertebrates for GM (Bt) cotton fields vs. non-pesticide controls, yet found little reductions in biodiversity in others. More recent research on aquatic environments has sparked intense interest in the impact of Bt-crops on aquatic invertebrates *Daphnia magna* (Bøhn et al. 2008), and caddisflies (Rosi-Marshall et al. 2007). These publications warrant future study, given the potential load of novel target proteins that may end up in agricultural runoff and end up in aquatic environments. Further, (Douville et al. 2007) present evidence of the persistence of the transgenic insecticidal protein Cry1Ab in aquatic environments and suggest that that sustained release of this potently bioactive compound from Bt maize production could result in negative impact on aquatic biodiversity.
Impacts on soil microflora and fauna, including earthworms (Zwahlen et al. 2003), mychorizzal fungi (Castaldini et al. 2005) and microarthropods in response to Cry endotoxins have also been reported (Wandeler et al 2002, Griffiths et al 2006, Cortet et al 2007).
The significance of tri-trophic effects of accumulation, particularly of insecticidal Cry toxins (Harwood et al. 2006, Obrist et al. 2006) is, however, yet to be firmly established. It has been demonstrated that sub-chronic dosages of Cry proteins may affect both foraging behavior and learning ability in non-target bees (Ramirez-Romero et al. 2008), and may have indirect effects

on recipient populations, and, given the key-stone role of bees as pollinators, on both primary production and on entire food-webs.

In relation to health impacts, a publication by (Dona and Arvanitoyannis 2009) reviews the potential health implications of GM foods for humans and animals, including incidences and effects of increased immunogenicity, amounts of anti-nutrients, possible pleiotropic and epigenetic effects, including possible reproductive and developmental toxicity. They conclude that while there is strong evidence for health concerns on many fronts, exposure duration many have not been long enough to uncover important effects. Studies should also include subjects with immunodeficiency or exposed to other stress agents.

Indications of harm to non-target organisms in the environment, and possible impacts to human and animal health prompted the Austrian Authorities to invoke a safeguard clause to ban the use of Cry1Ab-containing maize event MON810 (Umweltbundesamt, 2007). We refer to this report as a detailed analysis of potential adverse effects from a Cry1Ab-producing GMO.

Vegetative insecticidal proteins (Vip)
VIP is one of a number extracellular compounds, in addition to crystal-associated toxin polypetides, that may contribute to the virulence of *B. thurungensis* (Liu et al 2007). These proteins have a broad insecticidal spectrum, which includes activity against a wide variety of lepidopteran as well as coleopteran pests and they may represent a new generation of insecticidal toxins that could be efficacious against insects that are resistant to Cry toxins (Asokan et al 2012, Mahon et al 2012). In that regard, one strategy involves the presentation of several toxins together, especially if a differing mode of action involving different receptors is available (Meserati et al 2005).
The *vip3Aa19* gene, described in this stacked event, is a modified version of the native *vip3Aa1* gene (Estruch et al, 1996) found in the *Bacillus thuringiens* strain AB88. It encodes a Vip3Aa19 protein that differs from the Vip3Aa1 protein encoded by the *vip3Aa1* gene by a single amino acid at position 284. The *vip3Aa1* gene encodes lysine at position 284 and the *vip3Aa19* gene encodes glutamine.

In a review by van Frankenhuyzen (2013) on the cross-order activity of *Bacillus thuringiensis* proteins they found that activity of a number of these proteins was not restricted to particular insect orders as once thought. Although this study did not document cross-order activity of Vip3Aa proteins specifically, ‘lack of presence is not proof of absence’ as the author put it, indicating that much more work still has to be done before conclusions can be drawn (van Frankenhuyzen, 2013).

In this stack, there are two Cry proteins and one VIP protein. The VIP and Cry proteins seem to have the same target species. However, special concern or vigilance should be paid to GM stacks that combine events that have similar type of mode of action through their expressed transgenic proteins. Also, the Cry proteins can attach to the same receptor, changing their mode of action. In theory, the presence of two toxins can result in cross resistance and a changed effect on target and also non-target species (Schnepf et al 1998, Hua et al 2001, Estela et al 2004, Li et al 2004).

Recommendation:

- The potential for cross resistance between Cry- and VIP proteins and a changed effect on target and also non-target species should be investigated.
- The potential for non-target effects of VIP proteins should be investigated, and should include species not of the order Lepidoptera.
- The Applicant should survey for Vip3A resistance alleles prior to the use of this toxin.

Herbicides as co-products

Herbicide tolerant (HT) plants are specifically designed to be used in combination with herbicides, and will always be sprayed with the intended herbicide. Without spraying the introduction of HT plants would be useless. Surprisingly, these herbicides are often not tested as part of the assessment and risk evaluation of HT plants. In feeding studies with HT GM plants for quality assessment the herbicide is systematically overlooked, which represents a serious flaw in the testing and risk evaluation. Viljoen et al. (2013) found that in 13 out of 16 published feeding studies with HT GM crops the plant material used had not been sprayed with the intended co-technology herbicide. There is also a gap in knowledge regarding herbicide accumulation and residues, including metabolic pathways and metabolites thereof. Bøhn et al. (2014) documented high levels of glyphosate residues in HT GM soybeans grown in the USA, and the same research group have published papers showing that such residues negatively affect the feed quality of HT GM soybeans (Cuhra et al., 2015). Moreover, safety testing (in relation to health and environmental issues) has been focused on the active ingredient in the co-technology herbicides, and not the commercial formulations actually used, providing unrealistic and possibly misleading results (Mesnage et al., 2014). Stacked HT GM plants are tolerant to one or more agrochemicals, allowing for combinatory and alternating use of several herbicides. Tolerance to multiple herbicides is also often combined with multiple Cry proteins that could have additive or even synergistic effects on non-target species and the environment.

In the toxicology assessment it is not mentioned if the focus is only on the resulting proteins from the inserted genes, or if the potential of herbicide exposure through consumption of herbicide treated maize also is considered. A recent study found that glyphosate and AMPA, constituents of the herbicide Roundup accumulated in soybeans (Bøhn et al., 2014), highlighting the importance of including the herbicides in the comparative and toxicological assessment of GM crops with herbicidal co-technology.

Glyphosate tolerance

The *CP4-EPSPS* gene from *Agrobacterium sp. line CP4* is from the insert of parental line NK603 in the multistack. It confers tolerance to herbicides products containing glyphosate.

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

Glyphosate kills plants by inhibiting the enzyme 5-enolpyruvoyl-shikimate-3-phosphate synthase (EPSPS), necessary for production of important amino acids. Some microorganisms have a version of EPSPS that is resistant to glyphosate inhibition.

Recent studies indicate that agriculture of GM plants is associated with greater overall usage of pesticides than the conventional agriculture (Benbrook 2009). Large proportions of GM agriculture is glyphosate tolerant crops (GT-cultivars) (James 2010).
A restricted number of recent publications indicate unwanted effects of glyphosate on health (Dallegrave et al 2003, Malatesta M et al 2002), aquatic (Solomon K & Thompson D 2003) and terrestric (Ono MA et al 2002, Blackburn LG & Boutin CE 2003); organisms and ecosystems.
A study of Roundup effects on the first cell divisions of sea urchins (Marc J et al 2002) is of particular interest to human health. The experiments demonstrated cell division dysfunctions at the level of CDK1/Cyclin B activation. Considering the universality among species of the CDK1/Cyclin B cell regulator, these results question the safety of glyphosate and Roundup on human health. In another study (Axelrad JC et al 2003) it was demonstrated a negative effect of glyphosate, as well as a number of other organophosphate pesticides, on nerve-cell differentiation. Surprisingly, in human placental cells, Roundup is always more toxic than its active ingredient. The effects of glyphosate and Roundup were tested at lower non-toxic concentrations on aromatase, the enzyme responsible for estrogen synthesis (Richard S et al, 2005). The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation. The authors conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. They suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.

Additionally, the International Agency for Research on Cancer (IARC) recently released a report concluding that glyphosate is “probably carcinogenic to humans” (Fritschi et al., 2015).

Glufosinate-ammonium tolerance
The event 1507 in 1507 x MIR162 x MON810 x NK603 maize contain the *pat* gene from *Streptomyces viridochromogenes* that confers tolerance to herbicides containing glufosinate-ammonium, a class of herbicides that are banned in Norway and in EU (except a limited use on apples) due to both acute and chronic effects on mammals including humans. Glufosinat ammonium is harmful by inhalation, swallowing and by skin contact. Serious health risks may result from exposure over time. Effects on humans and mammals include potential damage to brain, reproduction including effects on embryos, and negative effects on biodiversity in environments where glufosinate ammonium is used (Hung 2007; Matsumura et al. 2001; Schulte-Hermann et al. 2006; Watanabe and Sano 1998). According to EFSA, the use of glufosinate ammonium will lead to exposures that exceed acceptable exposure levels during application.

Recommendation:

- The applicant should include a full evaluation of the co-technology intended to be used with 1507 x MIR162 x MON810 x NK603, namely glyphosate- and glufosinate ammonium based herbicides. Particular focus should be given to the level of accumulation of herbicides in the plants, particularly the parts used in food and feed production, and whether or not these levels of exposure could cause acute and/or chronic health issues.

Molecular characterization.
Based on the information from the summary of the technical dossier, it is not possible to see if the molecular characterization of the distinct inserts have been performed on the stack, or if th characterization is based on previous evaluations of the single, parental lines constituting the multistack in this Application.

Information about the nucleic acid(s) sequences actually inserted or deleted
Neither the source of the DNA, the vectors used or description of the different traits are present in the summary. The Applicant is referring to the assessments of each single event in this multistack, not considering potential changes or rearrangements upon combining them.

Recommendation:

- The Applicant is encouraged to characterize the distinct inserts as they are present in the multistack of this Application, and not based on previous characterizations of the single events.

According to the Applicant, the subcellular location of the inserts have been determined by Southern blots and flanking sequences investigated by BLAST search.
We can not comment on this data due to lack of access to the full technical dossier.

The organization of the inserted genetic material /insertion site have also been investigated by Southern blot and found equal to parental lines.

The e35S promoter
MON810 maize is one of the parental lines of the multistack in this application. This event contains the e35S promoter from Cauliflower Mosaic Virus.
Safety questions related to the use this promoter (P35S) in GM plants has been discussed in an article from Podevin and Du Jardin (2012). In this 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 (Shivaprasas 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.
In order to estimate the expression level of inserted proteins, the multistack 1507 x MON810 x MIR162 x NK603 was grown and analyzed in field studies. Protein levels were compared to GM parental lines accordingly, in key plant tissues at different developmental stages using enzyme-linked immune-sorbent assay (ELISA).

As we do not have access to the results of these studies or the study design for the ELISA, we can not comment on the antigens or antibodies used for the different analysis.

The Applicant do however comment that the levels of expression were comparable to the "corresponding GM parental lines".

Toxicology and allergenicity

The multistack 1507 x MON810 x MIR162 x NK603 express several different proteins encoded from its parental GM lines. This include Cry1F, Cry1Ab, Vip3Aa20, PAT and CP4 EPSPS.

According to the summary of the technical dossier, all proteins have been assessed for toxicity based on the following:

- History of safe use of the donor organism of the protein
- Toxicity risk based on molecular and biochemical characteristics
- Aminoacid sequence homology to known toxins or biologically active proteins causing adverse effects in humans or animals
- Acute oral toxicity in mammals

It is not clear from the summary of the technical dossier if it is the plant or the bacterial version of the distinct proteins that are used for the safety assessment.
According to the Applicant, no reports have shown that the proteins expressed in this GM multistack poses adverse effects on human or animal health.

The Applicant also states that there is no evidence for the potential of interactions between the different proteins encoded by the multistack. It is however not clear how the potential for these interactions have been investigated.

The proteins expressed in the multistack were also investigated for the potential of allergenicity through a weight of evidence approach assessing the potential for allergenicity of new proteins. They were evaluated through:

- Allergenic potential of source of genes
- Homology searches in allergen databases
- Incubation in simulated gastric fluid (in vitro)
- Analysis of glycosylation and heat stability.

There is however little information about the combination of proteins and allergy as they are expressed in this multistack.

Recommendation:

- We encourage the Applicant to specify the source of proteins used for safety analysis, also in the summary of the technical dossiers.
- We also encourage the Applicant to consider the combined expression of the distinct proteins in the whole plant in the analysis of toxicology and allergy.

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. In the following we identify issues that are relevant to consider in order to assess social utility and sustainability aspects, and highlight the need for further information to properly assess these issues.

<u>Impacts in producer countries</u>

The NGTA, with its clauses on societal utility and sustainable development, comes into play with a view also to health, environmental and socio-economic effects in other countries, such as where the GMOs are grown.

Social impact relevant for sustainability

Published reviews on sustainability-relevant aspects of social impacts from cultivating GM crops (e.g. impacts among poor and/or small-scale farmers in developing countries, share of the benefits among sectors of the society) indicate that these effects have been very complex, mixed and dependent on the agronomic, socio-economic and institutional settings where the technology has been introduced (Glover, 2010). Fisher et al. (2015) performed a literature review on empirical studies concerning social implications from cultivating GM crops, and found that from 2004 – 2015 there has only been 15 studies corning social implications of cultivating Bt-maize. They show that published literature is dominated by studies of economic impact and conclude that very few studies that take a comprehensive view of social impacts associated with GM crops in agriculture. Importantly, it is difficult to extrapolate on hazards or

risks taken from data generated under different ecological, biological, genetic and socio-economic 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. In order to meet the requirements in the NGTA, further investigations of social implications (e.g. economic, distribution of benefits, access to seeds and wellbeing) in countries where maize 1507 X MIR162 X MON810 X NK603 is intended for cultivation is needed.

Co-existence management
The cultivation of GM plants in general is causing problems with regard to co-existence. For instance, Binimelis (2008) have investigated consequences on co-existence of Bt maize in Spain among small-scale farmer and has found that co-existence is very difficult and that farmers in some areas has given up growing non-GM maize. Information about the strategies adopted to ensure co-existence with conventional and organic maize production and information about consequences on co-existence in the countries intended for production of maize 1507 X MIR162 X MON810 X NK603 is required.

Impacts of the Bt-toxin on target and non-target organisms in the producer country
The 1507 X MIR162 X MON810 X NK603 maize confers resistance to certain lepidopteran and coleopteran pests. A growing number of studies and reviews indicate potential harm to a range of non-target organisms (Holderbaum et al. 2015; Marvier et al. 2007; Rosi-Marshall et al. 2007; Bøhn et al. 2008). Both impacts on non-target organisms and resistance development among target pests of Bt maize has been documented (Van den Berg et al. 2013; Van den Berg, 2013). Evaluation of resistance development within the target pest population and strategies suggested to halt this development, as impacts on non-target organisms is crucial in a sustainability assessment.

Environmental and health impacts of the co-technology: glufosinate-ammonium and glyphosate
The evaluation of the co-technology, that is, secondary products that are intended to be used in conjunction with the GMO, is also considered important in the risk assessment of a GMO (Dolezel et al., 2009). Therefore, considerations of the co-products also warrant an evaluation of safe use.
The 1507 x MIR162 x MON810 x NK603 maize confers tolerance to herbicides containing glufosinate-ammonium and glyphosate. Glufosinate-ammonium is a class of herbicides that are banned in Norway and in the EU (except a limited use on apples) due to both acute and chronic effects on mammals including humans (see page 13 for references and further elaboration on this issue).

Recent studies have shown negative effects from glyphosate, both on species present in terrestrial and aquatic ecosystems and on animals and cell cultures. Consequently, glyphosate is now increasing recognized as more toxic to the environment and human health than what it was initially considered to be (see page 12-13 for references).
Emergence of herbicide resistant weeds in maize is vastly documented globally, particularly for glyphosate[1], and it is documented that the introduction of glyphosate tolerant GM plants has

[1] http://weedscience.org/summary/crop.aspx

led to an increase in the use of glyphosate (Dill et al. 2010). Moreover, studies has shown increased levels of glyphosate residues in glyphosate tolerant GM crops (Bøhn et al. 2014). This could have health impacts on humans and animals consuming food/feed based on ingredients from this type of GM plants.

The Applicant should provide information on the contribution of the 1507 x MIR162 x MON810 x NK603 maize to the emergence of glyphosate/glufosinate-ammonium resistance in weeds, management strategies to prevent herbicide resistance development in weeds, and if there are already cases of this in the areas intended for cultivation of the variety. In order to evaluate changes in the use of glyphosate/glufosinate-ammonium, after the introduction of 1507 x MIR162 x MON810 x NK603 maize, more information about the use of these herbicides in the producing country(ies) are needed.

Assessment of alternatives

It is also important to evaluate whether alternative options (e.g. the parental non-GM version of the 1507 x MIR162 x MON810 x NK603 maize) may achieve the same outcomes in a safer and ethically justified way. Furthermore, in order to evaluate whether the 1507 x MIR162 x MON810 x NK603 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. GM maize accounts for approximately 30% of the current global maize production (www.GMO-compass.org). Non-GM maize is therefore abundant for importation to the Norwegian market and maize 1507 x MIR162 x MON810 x NK603 can therefore not be considered to meet a societal need or demand.

Impacts of and ethical considerations in relation to the use of glufosinate-ammonium

While it is understood that the Applicant has not applied for deliberate release of the 1507 x MIR162 x MON810 x NK603 maize in Norway, the acceptance of a product in which the intended use involves the use of a product banned in Norway, as the glufosinate-ammonium, would violate basic ethical and social utility criteria, as laid out in the NGTA. Therefore we find that it would be ethically incongruous to support a double standard of safety for Norway on one hand, and safety for countries from which Norway may import its food and feed on the other. This line of reasoning is consistent with the provisions under the NGTA to assess ethical, social utility and sustainable development criteria not only for Norway, but for countries from which Norway imports food and feed. Specifically, this issue is relevant particularly in the revised guidelines for impact assessment pursuant to the Act of 2005 Section 17, "*Other consequences of the production and use of genetically modified organisms"* points 2 and 3, "*ethical considerations that may arise in connection with the use of the genetically modified organism(s)*», and "*any favorable or unfavorable social consequences that may arise from the use of the genetically modified organism(s)*", respectively.

Recommendation:

- In order to meet the requirements for the NGTA, the regulator is encouraged to ask the Applicant to submit information relevant for the assessment of the social utility of the 1507 x MIR162 x MON810 x NK603 maize and its contribution to sustainable development. The information provided by the Applicant must be relevant for the

agricultural context in the producing country/countries. The information should include issues such as: development of pest resistance in target populations, impacts on non-target organisms, herbicide resistance in weed populations, co-existence consequences and possible impacts among poor and/or small-scale farmers in producing countries and share of the benefits among sectors of the society.

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 1507 x MIR162 x MON810 x NK603 maize and does therefore not provide sufficient information as required by the NGTA.

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