Vår ref:2015/H_123
Deres ref.: 2015/5918

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 17.08.15

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på høringen av søknad **EFSA/GMO/NL/2014/123** fra Pioneer Hi-Bred International, Inc. som gjelder mat, fòr, import og prosessering av genmodifisert mais **4114.**

Vennligst ta kontakt hvis det er noen spørsmål.

Med vennlig hilsen,

Idun Merete Grønsberg
Forsker II
GenØk – Senter for Biosikkerhet
idun.gronsberg@genok.no

Bidragsytere:

Frøydis Gillund
Forsker II
GenØk – Senter for Biosikkerhet

Thomas Bøhn
Forsker I
GenØk – Senter for Biosikkerhet

Marianne Iversen
Forsker III
GenØk – Senter for Biosikkerhet

Vår ref:2015/H_123
Deres ref.: 2015/5918

Assessment of the summary of the technical dossier submitted under EFSA/GMO/NL/2014/123 for approval of 4114 maize.

Sent to

Norwegian Environment Agency

by

GenØk- Centre for Biosafety
August 2015

KONKLUSJON

Vi trekker frem mangler i oppsummeringen av dossieret som ikke gir grunnlag for en konklusjon om sikker bruk, samfunnsnytte og bidrag til bærekraft av **4114 mais**. Søker har ikke inkludert noe av den informasjonen omkring samfunnsnytte og bærekraft av **4114 mais** som kreves i den norske genteknologiloven (Appendix 4) for godkjenning i Norge.

Hovedkonklusjon og anbefalinger:
Genøk–Senter for Biosikkerhet viser til brev fra Miljødirektoratet angående høring som omfatter **4114 mais** for bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **4114 mais.**

Søker gir ikke opplysninger som adresserer vurderingskriteriene bærekraft, samfunnsnytte og etiske aspekter som forutsettes anvendt i den norske genteknologiloven. 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 oppsummeringen av søknaden om omsetting av **4114 mais** til import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **4114 mais.**

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av **4114 mais** til import og prosessering og til bruk i fòr og mat som det søkes om.

Vår ref:2015/H_123
Deres ref.: 2015/5918

ASSESSMENT OF THE SUMMARY OF TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL/2014/123 FOR APPROVAL OF 4114 MAIZE.

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 **4114 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

- The regulator is encouraged to ask the applicant to address the potential of non-target effects of Bt toxins, especially in the context of their combined use in a stacked event.
- The regulator is encouraged to ask the Applicant to consider the possibility of cross resistance development to multiple Cry proteins due to the use of stacked events.
- We find it ethically unacceptable to ban the use of glufosinate-ammonium based herbicides domestically due to health and environmental concerns, while supporting its use in other countries. This represents an unacceptable double standard for Norway, and we ask the regulators to reconsider the practice of separating health and environmental risk by national borders or regions
- The applicant should include a full evaluation of the co-technology intended to be used with 4114, namely 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. This needs to be tested in animal and feeding studies, separating the effects of the plant and the herbicide(s) by using both sprayed and unsprayed plant samples.
- The Applicant should look into and compare the levels of herbicide residues in the plants in order to provide an improved comparative assessment. The health implications (if any) of the herbicide residue exposure to humans and animals should subsequently be discussed in the toxicological assessment. The toxicological assessment should also include a section on farm worker exposure to the herbicide.
- The Applicant should use herbicide treated, as well as untreated plant material in long-term chronic exposure feeding studies.
- The environmental risk assessment should include a section on the potential environmental effects of the herbicide (monitoring changes in use, potential drift into surrounding areas and ecosystems, leaching to aquatic environments, potential effects on wildlife).
- The regulators are encouraged to ask the Applicant to provide a full ERA of the life cycle of 4114 from being planted in the field and through the cultivation process, harvesting, transportation, processing, and as waste. Specifically, more information on risk management with regards to gene flow and herbicide regime should be included in the ERA.
- The regulator is encouraged to ask the Applicant to 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.
- The regulator is encouraged to ask the applicant to address the potential of non-target effects of Bt toxins, especially in the context of their combined use in a stacked event.
- The regulator is encouraged to ask the Applicant to consider the possibility of cross resistance development to multiple Cry proteins due to the use of stacked events.
- The regulator is encouraged to ask the Applicant to provide a full technical dossier to be able to comment on any of the molecular data claimed in the summary of the technical dossier.
- 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

4114 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: Changes in pesticide use, emergence of herbicide resistant weeds, development of pest resistance in target populations, impacts on non-target organisms, potential for gene flow 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

From our analysis, we find that the deficiencies in the summary of the dossier do not support claims of safe use, social utility and contribution to sustainable development of 4114 maize**. 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.** 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 **4114 maize**, we conclude that based on the available data, the Applicant has not provided the required information under Norwegian law to warrant approval in Norway at this time.

ASSESSMENT OF THE SUMMARY OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL/2014/123

About the event

4114 maize was produced by *Agrobacterium tumefaciens* mediated transformation of a Pioneer proprietary maize line.

Expression cassettes for Cry1F, Cry34Ab1, Cry35Ab1 and PAT were isolated from plasmids used in maize event 1507 and 59122.

This stacked event produces the proteins Cry1F, Cry34Ab1 and Cry35Ab1 to provide insect tolerance. The proteins provide protection from feeding damage caused by certain lepidopteran and coleopteran pests.

The event also produces PAT to provide tolerance to the herbicide glufosinate ammonium.

This application is for food, feed, processing and import. Application for full range use, including cultivation, have been made to USA, Canada and Japan. In addition, approval for the applications for food and feed have been sent to countries with regulatory approval systems.

Assessment findings

Safety of Cry genes

Maize 4114 combines different classes of Bt proteins named Cry toxins, namely Cry1F, Cry34Ab1 and Cry35Ab1. These toxins are claimed to be safe, however the potential of non-target effects of Bt toxins, including alternative modes of action for Cry toxins have been addressed previously (Bøhn et al 2008 , Gilliand et al 2002, Crickmore 2005, Hilbeck and Schmidt 2006)).

Negative effects of Bt-transgenic plants on non-target organisms are documented. A meta-analysis of published studies on non-target effects of Bt-proteins in natural enemies, (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. Further, Cry toxins and proteinase inhibitors have often non-neutral effects on natural enemies, and more often negative than positive effects. A review by Hilbeck and Schmidt (2006) on Bt-plants, found 50% of the studies documenting negative effects on tested invertebrates.

Additionally, a recent review by van Frankenhuyzen (2013) indicated that several Cry proteins exhibit activity outside of their target orders. This study also found that many Cry proteins only had been tested with a very limited number of organisms: thus, activity outside of the target organisms of many Cry proteins may be undocumented simply because testing has not included sensitive organisms (van Frankenhuyzen, 2013). As not every potentially sensitive species can be tested for sensitivity to Bt toxins, it cannot be excluded that sensitive species have been overlooked in testing until now. The issue is complicated further by the number of variables which can affect toxicity testing, which may include toxin preparation and purification, life stage of the specimens, differences in toxin expression hosts, as well as solubilization (or lack thereof) of the toxin, among other factors (van Frankenhuyzen 2009).

A quantitative review analysis based on 42 field experiments showed that unsprayed fields of Bt-transgenic maize plants have significantly higher abundance of terrestrial non-target invertebrates than sprayed conventional fields (Marvier et al. 2007). Thus, Bt-plants with a single Bt-gene inserted may represent an improvement for non-target organisms in the environment. However, an indication of some negative effects of the Cry1Ab toxin itself, or the Cry1Ab maize plant, on non-target abundance was shown in the same meta-analysis: when conventional (non-GM) fields were not sprayed, the non-target abundance was significantly higher than in the Bt-fields (Marvier et al. 2007).

Research on aquatic environments has sparked intense interest in the impact of Bt-crops on aquatic invertebrates including *Daphnia magna* (Bøhn et al 2008) and caddisflies (Rosi-Marshall et al 2007). Given the potential load of Cry toxins (also in combination with herbicides) that may end up in aquatic environments, further studies are warranted. Douville et al. (2007) presented evidence of the persistence of the *cry1Ab* transgene in aquatic environments: more than 21 days in surface waters, and 40 days in sediments. A follow-up on this study in 2009 indicated possible horizontal gene transfer of transgenic DNA fragments to aquatic bacteria (Douville et al 2009).

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.

The use of multiple, related transgenes in a single (stacked) event may accelerate resistance development to both transgene products. This was the experience of Zhao et al (2005), who tested the effect of using broccoli plants containing Cry1Ac, Cry1C or both, on resistance development in a population of diamondback moths (*Plutella xylostella*). They found that the stacked use of similar Cry proteins in close proximity to single gene events led to accelerated resistance development to both traits (Zhao et al 2005). Bravo and Soberón (2008) commented on this effect, acknowledging that gene stacking is not a universal solution to resistance development to Cry proteins. Studies such as these beg the question as to whether the stacked use of related Cry proteins, such as Cry1Ab and eCry3.1Ab, in the same event is advisable. In this application, stacks of Cry1F, Cry34Ab1 and Cry35Ab1 is present, together with an other insert.

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.

A recent study in mice showed that exposure to purified Cry1Ab resulted in specific anti-Cry1Ab IgG1 and IgE production, indicating inherent immunogenicity and allergenicity. Further, mice exposed to leaf extracts from both MON810 and unmodified maize demonstrated influx of lymphocytes and eosinophils in the broncho-alveolar lavage,and increased cytokine release in mediastinal lymph node cells (Andreassen et al 2015). Further studies should also include animals with immunodeficiencies and/or animals exposed to other stress agents simultaneously.

The potential adjuvancy of Cry proteins has previously been addressed by the GMO Panel of the Norwegian Scientific Committee for Food Safety. Also scientific studies have shown that the Cry1Ac protein is a potent systemic and mucosal adjuvant (Moreno-Fierros et al, 2003). In the evaluation of another GM maize, MIR604 x GA21, the panel found that it was difficult to evaluate if kernels from this stack would cause more allergenic reactions than kernels from unmodified maize. The Panel continues with: "*As the different Cry proteins are closely related, and in view of the experimental studies in mice, the GMO Panel finds that the likelihood of an increase in allergenic activity due to Cry1Ab and mCry3A proteins in food and feed from maize*

Bt11 x MIR604 x GA21 cannot be excluded. Thus, the Panel's view is that as long as the putative adjuvant effect of Cry1Ab and mCry3A with reasonable certainty cannot be excluded, the applicant must comment upon the mouse studies showing humoral antibody response of Cry1A proteins and relate this to a possible adjuvant effect of the Cry1Ab and mCry3A proteins expressed. Furthermore, although Cry1Ab and mCry3A proteins are rapidly degraded in gastric fluid after oral uptake, there is also the possibility that the protein can enter the respiratory tract after exposure to e.g. mill dust. Finally, rapid degradation is no absolute guarantee against allergenicity or adjuvanticity" (EFSA/GMO/UK/2007/48, Norwegian Scientific comitee for Food Safety, 12/06-08).
We also agree with these concerns.

Recommendation:

- The regulator is encouraged to ask the applicant to address the potential of non-target effects of Bt toxins, especially in the context of their combined use in a stacked event.
- The regulator is encouraged to ask the Applicant to consider the possibility of cross resistance development to multiple Cry proteins due to the use of stacked events.

Herbicide tolerance traits:

Herbicide tolerant (HT) plants are specifically designed to be used in combination with herbicides, and as such will always be sprayed with the intended herbicide. Without spraying there is no meaning to the introduction of HT. 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 large 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., 2014, Cuhra et al., 2015). Moreover, safety testing (in relation to health and environmental issues) has been focused on only 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, Surgan et al., 2010). 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 often combined with multiple Cry proteins, that could have additive or even synergistic effects on non-target species and the environment.
Though the application in question does not encompass the cultivation of event 4114, it must be mentioned that we are of the opinion that the environmental effects of the herbicide, as an important co-technology and essential part of the cultivation of this event, should be discussed in the environmental risk assessment.

Glufosinate-ammonium

The *pat* gene derived from *Streptomyces viridochromogens* confers tolerance to herbicides containing glufosinate-ammonium, a class of herbicides that is banned in Norway and in EU (except a limited use on apples) due to both acute and chronic health effects on mammals including humans. Studies have shown that glufosinate-ammonium is harmful by inhalation, ingestion and skin contact. Serious health risks may result from exposure over time. Observations of patients poisoned by glufosinate-ammonium have found that acute exposure causes convulsions, circulatory and respiratory problems, amnesia and damages to the central nervous system (CNS) (Watanabe 1998). According to EFSA, the use of glufosinate-ammonium will lead to farm workers being exposed to herbicide levels that exceed acceptable exposure levels during application.

Since the purpose of the insertion of the *pat* gene is to be able to treat the maize crop with glufosinate-ammonium based herbicides, we find it disconcerting that the presence of the herbicide has not been considered in the comparative assessment nor the toxicological assessment. Though the plant material used for the comparative assessment consisted of both herbicide treated and untreated plants the applicant has not tested the plant material for herbicide residues. In the toxicology assessment the applicant only focuses on the resulting proteins from the inserted genes, and do not discuss the potential of herbicide exposure through consumption of herbicide treated maize. 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 herbicide in the comparative and toxicological assessment of GM crops with herbicidal cotechnology.

Recommendation:

- We find it ethically unacceptable to ban the use of glufosinate-ammonium based herbicides domestically due to health and environmental concerns, while supporting its use in other countries. This represents an unacceptable double standard for Norway, and we ask the regulators to reconsider the practice of separating health and environmental risk by national borders or regions
- The applicant should include a full evaluation of the co-technology intended to be used with 4114, namely 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. This needs to be tested in animal and feeding studies, separating the effects of the plant and the herbicide(s) by using both sprayed and unsprayed plant samples.

Specific recommendations:
-The Applicant should look into and compare the levels of herbicide residues in the plants in order to provide an improved comparative assessment. The health implications (if any) of the herbicide residue exposure to humans and animals should subsequently be discussed in the toxicological assessment. The toxicological assessment should also include a section on farm worker exposure to the herbicide.
-The Applicant should use herbicide treated, as well as untreated plant material in long-term chronic exposure feeding studies.

-The environmental risk assessment should include a section on the potential environmental effects of the herbicide (monitoring changes in use, potential drift into surrounding areas and ecosystems, leaching to aquatic environments, potential effects on wildlife).

Environmental risk assessment (ERA) and monitoring plan

Though the ERA and monitoring plan in this dossier is mainly concerned with potential exposure of GM plant material to the environment in other ways than cultivation (the application does not encompass cultivation in Europe), 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).

Gene flow could have implications for insect life if cry-genes spread to wild maize relatives, or for herbicide resistance in wild maize relatives if genes such as *pat* or *cp4 epsps* are introduced. High doses and continuous use of only a few herbicides promotes development of resistance in weed species, creating a snowball effect where dosage continues to increase in order to overpower the weeds resistance mechanisms. The herbicide is never only confined to the field but will affect surrounding areas such as meadows and small forest. Additionally, if the herbicide persist for some time in nature in can leach from the field and into aquatic systems affecting the organisms living there.

The Norwegian Gene Technology Act §1 specifically states that "*The purpose of this Act is to ensure that the production and use of genetically modified organisms and the production of cloned animals take place in an ethically justifiable and socially acceptable manner, in accordance with the principle of sustainable development and without adverse effects on health and the environment*". We find that it would be double standard and poor ethical judgment to condone the import and use of crops, without knowing the agricultural context in which these crops are produced, and what steps that are being taken by producers to minimize risk and ensure a sustainable production with minimal impact on the environment and health of workers and consumers. Information on what measures are being taken to minimize the risk of gene flow to wild relative, and on the herbicide regime is essential for evaluating the sustainability and environmental impact of this crop. Thus, we would like for an ERA considering the risks connected also to cultivation of the crop in question to be included in the dossier.

Recommendation:

- The regulators are encouraged to ask the Applicant to provide a full ERA of the life cycle of 4114 from being planted in the field and through the cultivation process, harvesting, transportation, processing, and as waste. Specifically, more information on risk management with regards to gene flow and herbicide regime should be included in the ERA.

Stacked 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 than single gene events. It has been an increased interest for 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 within stacked traits cannot be excluded and that the group of expressed toxins in the plant can give specific immunological effects or adjuvant effects in mammals (Halpin 2005, deSchrijver 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.

The safety assessment of this application is **based on the summary of a technical dossier** as the full dossier is not available as of yet. Most of the information submitted in this safety assessment is derived from previous finding with the proteins in maize events 1507, 59122 and 1507 x 59122. The applicant has not demonstrated that interactions among the different transgenic proteins, particularly for allergenic or toxic effects, are not taking place in this event .Assumptions-based reasoning based on proteins expressed in other events rather than the event. in question should not replace scientific testing of hypotheses regarding interactions. GenØk means that stacked events cannot be approved based on the information from other events than the one applied for.

Maize 4114 combines several classes of Bt proteins active against insects pest like Lepidoptera and Coleoptera. 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.

Recommendation:

- The regulator is encouraged to ask the Applicant to 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.

Molecular characterization

3.1 Information relating to the genetic modification (p 11 in Summary)
Maize event 4114 has been produced by *Agrobacterium tumefaciens* mediated transformation of a maize line. The inserts in this stack are claimed to be the same as the expression casettes present in maize lines 1507 and 59122 expressing the proteins Cry1F, Cry34Ab1, Cry35Ab1 and PAT.
The applicant do not provide data in this summary of the dossier that can verify these claims or any other molecular data.

3.2.3 Information on the expression of the inserted/modified sequences (p.12 in Summary)
Expression levels of Cry1F, Cry34Ab1, Cry35Ab1 and PAT were analysed using ELISA in "key plant tissues". Since we do not have access to the full dossier, we can not comment on variation in expression levels or which tissues that have been analysed.

4. Comparative analysis (p. 13 in Summary)
The stacked maize event 4114 was compared to a non-GM near isogenic line. Data on commercial non-GM maize hybrids have also been used for comparisons.

Key nutrients and components were selected for analysis based on guidance provided by OECD for maize.
No data are available as the full dossier is not accessible. We can therefore not comment on the "few analytes that showed statistically significant differences" as we do not know which nutrients and at what level they were different.

4.5 Effects of processing
No data on this section is available.

5. Toxicology
The stacked event produces Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in the same plant. As no reports on potential adverse effects to human and animals have appeared, and re-analysis of the similarity searches (Bioinformatics) revealed no data negative to safety, the proteins are claimed to be safe.
As the summary of the dossier only is available, we can not comment on what version of the proteins that have been used for the studies that have been performed. The applicant refers to data of proteins expressed in 1507 x 59122 maize as they express the same proteins as the 4114 maize in this application.

We recommend the applicant to use proteins isolated from the authentic GM plant (the stack) and use this for the analysis as the proteins are expressed in a different context in this stack than in the one referred to in the summary of the dossier.

Data on acute oral toxicity or repeated dose toxicity is not available and not mentioned in the summary of the technical dossier.

6. Allergenicity

The proteins expressed in the stacked maize event 4114 is claimed to be tested in a "weight of evidence" approach.
There is no data available in the summary of the technical dossier on any of the factors tested in this approach. We can not comment on the allergenicity of the proteins expressed based on this.

Recommendation*:*

- We ask the Applicant to provide a full technical dossier to be able to comment on any of the molecular data claimed in the summary of the technical dossier.

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 areas that are relevant to consider in order to assess social utility and sustainability aspects, and highlight information that that is missing from the Applicant.

Impacts in producer countries

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 the 4114 maize is not yet approved for cultivation in any third country.

The Applicant does not provide data relevant for an ERA of the 4114 maize (as it is not intended to be cultivated in the EU/Norway). This information is necessary in order to assess the sustainability criteria as laid down in the NGTA. 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. It can therefore not be expected that the same effects will apply between different environments and across continents. Hence, a proper evaluation of potential impacts of relevance to sustainability cannot be completed until this event has been approved for cultivation in a third country, and sufficient information relevant for the ERA and socio economic impacts assessment in these agricultural contexts has been provided. This must include information from an ERA concerning impacts on cultivation, management and harvesting stages, as well as the post-market environmental monitoring in the producing country. With regard to potential socio-economic impacts in the producer country or countries, published reviews on sustainability-relevant aspects (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). The applicant does not provide any references to the extensive literature concerning the socio-economic aspects related to the cultivation (and to a much lesser extend, the use) of GM maize.

Impacts of the co-technology: glyphosate and glufosinat-ammonium

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 and data required for such an assessment is, as already described previously., and not provided by the Applicant. The 4114 maize confers tolerance to herbicides containing glufosinate-ammonium. 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 section on Herbicide tolerance for references and further elaboration on this issue) Weed resistance in maize cultivation has been vastly documented[1]. The Applicant has not provided information on the contribution of the 4114 maize to the emergence of glufosinate-ammonium resistance in weeds, nor if there are already cases of this in the areas intended for cultivation of the variety.

Impacts of the Bt-toxin on target and non-target organisms

The 4114 maize does also confer resistance to certain lepidopteran and coleopteran pests. A growing number of studies and reviews indicate potential harm to a range of non-target organisms (Marvier et al. 2007; Rosi-Marshall et al. 2007; Bøhn et al. 2008; Bøhn et al. 2010).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 well as impacts on non-target organisms is crucial in a sustainability assessment.

Impacts from gene flow and co-existence management

An evaluation of the occurrence of volunteer plants in the producing countries and suggested control strategies is important for a sustainability assessment. Information about the occurrence of volunteers and which herbicides that will potentially be used for killing volunteers is required to evaluate potential health and environmental impacts of these. The Applicant should describe strategies to ensure co-existence with conventional and organic maize crops in the producing countries and minimize the likelihood for gene flow to wild relatives.

[1] http://www.weedscience.org/Summary/Crop.aspx?SituationID=8

Assessment of alternatives

It is also important to evaluate whether alternative options (e.g. the parental non-GM version of the 4114 maize) may achieve the same outcomes in a safer and ethically justified way. Furthermore, in order to evaluate whether the 4114 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.

Ethical considerations

While it is understood that the Applicant has not applied for deliberate release of the 4114 maize in Norway, the acceptance of a product in which the intended use involves the use of a product banned in Norway, as the glyphosinate-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 4114 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: Changes in pesticide use, emergence of herbicide resistant weeds, development of pest resistance in target populations, impacts on non-target organisms, potential for gene flow 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 4114 maize is tolerant to glufosinate-ammonium which is banned for use in Norway. Banning the use of glufosinate-ammonium based herbicides domestically due to health and environmental concerns, while supporting its use in other countries would be ethically unacceptable. 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 4114 maize and does therefore not provide sufficient information as required by the NGTA.

References

Andreassen M., Rocca E., Bøhn T., Wikmark, OG., van Den Berg J., Løvik M., Traavik T and Nygaard UC (2015). Humoral and cellular immune responses in mice airway administration of Bacillus thuringiensis Cry1Ab and MON810 Cry1Ab –transgenic maize. Food and Agricultural Immunology, 26, pp. 521-37.

Bravo, A, Soberón, M (2008). How to cope with insect resistance to Bt toxins? Trends in Biotechnology, 26(10), pp. 573-579.

Bøhn, T., Primicerio, R., Hessen, D. O. Traavik. T., (2008). Reduced fitness of *Daphnia magna* fed a Bt-transgenic maize variety. Archives of Environmental Contamination and Toxicology, 55, pp.584-592.

Bøhn T., Cuhra M., Traavik T., Sanden M., Fagan J. and Primicerio R (2014). Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. Food Chemistry, 153**,** pp.207-215.

Castaldini M, Turrini A, Sbrana C, Benedetti A, Marchionni M, Mocali S, Fabiani A, Landi S, Santomassimo F, Pietrangeli B, Nuti M P, Miclaus N, Giovanetti M (2005). Impact of Bt corn on rhizospheric and soil eubacterial communities and on beneficial mycorrhizal symbiosis in experimental microcosms. Applied and Environmental Microbiology, 71, pp.6719-6729.

Cortet J, Griffiths BS, Bohanec M, Demsar D, Andersen M N, Caul S, Birch ANE, Pernin, C, Tabone, E, de Vaufleury A, Ke X, Krogh PH (2007). Evaluation of effects of transgenic Bt maize on microarthropods in a European multi-site experiment. Pedobiologia, 51, pp.207-218

Crickmore N, (2005). Using worms to better understand how Bacillus thuringiensis kills insects. Trends in Microbiology, 13(8), pp.347-350.

Cuhra M., Traavik T. and Bøhn T (2014). Life cycle fitness differences in Daphnia magna fed Roundup-Ready soybean or conventional soybean or organic soybean. Aquaculture Nutrition doi: 10.1111/anu.12199. pp1-12.

Cuhra M., Traavik T., Dando M L., Primicerio R., Holderbaum D F and Bøhn T (2015). Glyphosate-Residues in Roundup-Ready Soybean Impair Daphnia magna Life-Cycle. Journal of Agricultural Chemistry and Environment, 4 pp. 13.

deSchrivjer A., Devos Y., Van Den Bulcke M., Cadot P., De Loose M., De Reheul D and Sneyers M. (2007). Risk assessment of GM stacked events obtained from crosses between GM events. Trends in Food Science and Tecnology. 18, pp. 101-09.

Dona, A. & Arvanitoyannis, IS (2009). Health risks of genetically modified foods. Critical Reviews in Food Science and Nutrition, 49, pp.164-175.

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.

Douville M, Gagne F, Blaise C, Andre C, (2007) Occurence and persistence of Bacillus thuringiensis (Bt) and transgenic Bt corn cry1Ab gene from an aquatic environment. Ecotoxicology and Environmental Safety, 66, pp.195-203.

Douville M, Gagné F, André C, Blaise C, (2009). Occurrence of the transgenic corn cry1Ab gene in freshwater mussels (Elliptio complanata) near cornfields: Evidence of exposure by bacterial ingestion. Ecotoxicology and Environmental Safety, 72, pp.17-25.

Griffiths BS, Caul S, Thompson J, Birch A N E, Scrimgeour C, Cortet, J., Foggo A, Hackett CA, Krogh PH, (2006). Soil microbial and faunal community responses to Bt maize and insecticide in two soils. Journal of Environmental Quality, 35, pp.734-741

Gilliland A, Chambers CE, Bone E J, Ellar DJ (2002). Role of *Bacillus thuringiensis* Cry1 delta endotoxin binding in determining potency during lepidopteran larval development. Applied and Environmental Microbiology, 68, pp.1509-1515.

Glover D (2010). Exploring the Resilience of Bt Cotton's 'Pro-Poor Success Story'. Development and Change, 41, pp. 955–981.

Halpin, C (2005). Gene stacking in transgenic plants – the challenge for the 21st century plant biotechnology. Plant Biotechnology Journal, 3, pp. 141-55.

Harwood JD, Samson R.A, Obrycki, JJ (2006). No evidence for the uptake of Cry1Ab Btendotoxins by the generalist predator Scarites subterraneus (Coleoptera:Carabidae) in laboratory and field experiments. Biocontrol Science and Technology, 16, pp.377-388.

Hilbeck, A. & Schmidt, J. E. U., (2006). Another view on Bt proteins - how specific are they and what else might they do? Biopesticides International, 2, pp.1-50.

Lövei GL & Arpaia S (2005). The impact of transgenic plants on natural enemies: a critical review of laboratory studies. Entomologia Experimentalis et Applicata, 114, pp.1-14.

Marvier M., McCreedy C., Regetz J. and Kareiva P (2007). A Meta-analysis of effects of BT cotton and maize on non-target invertebrates. Science, 316, pp. 1475-77.

Mesnage R., Defarge N., Spiroux deVendemois J and Seralini GE (2014). Major Pesticides Are More Toxic to Human Cells Than Their Declared Active Principles. BioMed Research International, 2014, 8.

Moreno-Fierros L., Ruiz-Medina EJ., Esquivel R., Lopez-Revilla R. and Pina-Cruz, S (2003). Intranasal Cry1Ac protoxin is an effective mucosal and systemic carrier and adjuvant of

Streptococcus pneumonia polysaccharides in mice. Scandinavian Journal of Immunology, 57, pp. 45-55.

Obrist LB, Dutton A, Romeis J, Bigler F (2006). Biological activity of Cry1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator *Chrysoperla carnea*. Biocontrol, 51, pp.31-48.

Ramirez-Romero R., Desneux N, Decourtye A, Chaffiol A, Pham-Delegu, MH (2008). Does CrylAb protein affect learning performances of the honey bee *Apis mellifera* L. (Hymenoptera, Apidae)? Ecotoxicology and Environmental Safety, 70, pp.327-333.

Rosi-Marshall, E.J., Tank JL, Royer TV, Whiles MR, Evans-White M., Chambers, C., Griffiths NA., Pokelsek J. and Stephen ML (2007). Toxins in transgenic crop byproducts may affect headwater stream ecosystems. Proceedings of the National Academy of Sciences of the United States of America 104, pp. 16204-16208.

Surgan M., Condon M and Cox C. (2010). Pesticide Risk Indicators: Unidentified Inert Ingredients Compromise Their Integrity and Utility. *Environmental Management*, 45**,** pp 834-841.

Then C (2009). Risk assessment of toxins derived from Bacillus thuringiensis – synergism, efficacy and selectivity. Environ Sci Pollut Res,17, pp. 791-7.

van Frankenhuyzen K (2009) Insecticidal activity of Bacillus thuringiensis crystal proteins. Journal of Invertebrate Pathology, 101, pp. 1-16.

van Frankenhuyzen K (2013). Cross-order and cross-phylum activity of *Bacillus thuringiensis* pesticidal proteins. Journal of Invertebrate Pathology, 114, pp. 76-85.

Viljoen, C. (2013). Letter to the editor. Food Chem Toxicol, 59**,** pp.809-10.

Wandeler, H., Bahylova, J., Nentwig, W., (2002). Consumption of two Bt and six non-Bt corn varieties by the woodlouse *Porcellio scaber*. Basic and Applied Ecology, 3, pp.357-365.

Watanabe T and Sano T (1998) Neurological effects of glufosinate poisoning with a brief review. Human & Experimental Toxicology 17:35-39.

Warwick S. I., Beckie H J.and Hall LM (2009). Gene Flow, Invasiveness, and Ecological Impact of Genetically Modified Crops. Ann NY Acad Sci, 1168**,** pp.72-99.

Zhao J-Z, Cao J, Collins H, Bates S L, Roush R T, Earle E D, Shelton A, (2005). Concurrent use of transgenic plants expressing a single and two Bacillus thuringiensis genes speeds insect adaptation to pyramided plants. Proceeding of the National Academy of Sciences, 102(24), pp. 8426-8430.

Zwahlen C., Hilbeck A., Howald R. and Nentwig W. (2003) Molecular Ecology. 12, pp. 1077-86.