Vår ref:2015/H_117
Deres ref.: 2015/5920

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 17.08.15

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på høringen av søknad **EFSA/GMO/BE/2013/117** fra Monsanto Company som gjelder mat, fòr, import og prosessering av genmodifisert mais **MON87427 x MON89034 x NK603**.

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

Hermoine Jean Venter
Forsker
GenØk – Senter for Biosikkerhet

Vår ref:2015/H_117
Deres ref.: 2015/5920

Assessment of the technical dossier submitted under EFSA/GMO/BE/2013/117 for approval of MON87427 x MON89034 x NK603 maize.

Sent to

Norwegian Environment Agency

By

GenØk- Centre for Biosafety
August 2015

KONKLUSJON PÅ NORSK

Vi trekker frem mangler i dossieret som ikke gir grunnlag for en konklusjon om sikker bruk, samfunnsnytte og bidrag til bærekraft av **MON87427 x MON89034 x NK603 mais**. Søker har ikke inkludert noe av den informasjonen omkring samfunnsnytte og bærekraft av **MON87427 x MON89034 x NK603mais** 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 **MON87427 x MON89034 x NK603 mais** for bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **MON87427 x MON89034 x NK603 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 søknaden om omsetting av **MON87427 x MON89034 x NK603 mais** til import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **MON87427 x MON89034 x NK603mais.**

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av **MON87427 x MON89034 x NK603 mais** til import og prosessering og til bruk i fòr og mat som det søkes om.

ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/BE/2013/117 FOR APPROVAL OF MON87427 x MON89034 x NK603 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 **MON87427 x MON89034 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.

We have previously commented on sub-combinations and single events of **MON87427 x MON89034 x NK603 maize** in:

- EFSA/GMO/NL/2009/72 for MON89034 x NK603 (our previous comments from January 2010: **H_72**)

- EFSA/GMO/BE/2011/90 for MON89034 (our previous comments from July 2012: **H_90**)

We have also commented on other combinations of the events (in other stacks) in this Application previously.

Specific recommendations

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

- 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 applicant should include a full evaluation of the co-technology intended to be used with MON87427xMON89034xNK603, namely glyphosate-based herbicide. 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 regulators are encouraged to ask the applicant to provide a full ERA of the life cycle of MON87427xMON89034xNK603 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 (even when the application does not include cultivation).
- 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 provide missing figures, match figure texts (Figure 8 and 9) and add molecular weight markers to Southern blots to be able to verify fragment sizes in the molecular characterization.
- The applicant should perform longer exposure times for Southern blots to be able to render more prominent bands darker and being able to see more faint bands so that all bands are potentially visible.
- The use of large probes can reduce the ability to detect changes in the gene sequence. We encourage the applicant to also use shorter probes.
- New profiling techniques can be used to detect inserts and look for deletions or alterations of sequences. We encourage the applicant to make use of these.
- We suggest that the Applicant perform toxicity studies with plant derived proteins from the stack the Applicant applies authorization for here.
- We encourage the Applicant to analyse proteins isolated from the stacked event to investigate proteins as they are expressed in the plant, and not base safety assessments on data from single events and stacks where proteins are expressed in another context.
- 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 MON87427xMON89034xNK603 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.

Our previous recommendations/points on sub-combinations and single events of **MON87427 x MON89034 x NK603.**

Application: EFSA/GMO/NL/2009/72 for MON89034 x NK603 (our previous comments from January 2010: H_72):

"Conclusion Based on the above, and with special attention on the independent evaluation of safety data with NK603, confidence in the safety of this maize variety (MON 89034 x 1507 x NK603) is scientifically unjustified at this time. Further evidence of lack of harm, including follow up feeding studies of longer duration and higher statistical power are needed. Therefore, in our assessment of MON 89034 x NK603 we conclude that based on the available data, including the safety data supplied by the producer, is insufficient and equivocal in its proof of lack of toxicological affects on mammalian health. We find that these effects may be biologically significant and warrant future study before claims of lack of harm can be scientifically established".

Application: EFSA/GMO/BE/2011/90 for MON89034 (our previous comments from July 2012: H_90):

Recommendations:

- The Applicant should re-perform the analysis using more sensitive methods (greater Escore sensitivities and smaller search sequence lengths) as well all relevant maize databases that maximize the likelihood of finding a accurate nucleotide match.
- The Applicant should report the nucleotide sequences obtained from the endogenous maize DNA regions adjacent to the transgene insertion(s) so that independent analysis can be performed.
- The Applicant should provide information demonstrating the genetic similarity of comparators used in comparative assessments in accordance with Regulation (EC) No 1829/2003 and Directive 2001/18/EC.
- The Applicant should provide a case-specific monitoring plan to monitor potential unintended but anticipated exposure routes and levels, and to verify the assessment of exposure routes and levels into the environment.
- The Applicant should provide more detail on the methods, locations and local considerations that should be identified for the establishment of baseline data.
- The Applicant should describe how the monitoring report would review and evaluate the effectiveness, relevance, efficiency, and scientific quality of data derived from monitoring, including the continuity of the monitoring activities as it was described in the monitoring plan. Any unusual observations or identified adverse effects that is identified should be reported in a timely manner so that the appropriate response may be undertaken. These reports should also include a scientifically rigorous analysis of the results and conclusions, also considering site-specific conditions. The report should further highlight results that indicate adaptation of the monitoring plan, further research or review of risk management options or decisions.

- The Applicant should also specify how they're report will provide information on the practical experience from the monitoring and suggest the ways the plan may be revised as needed, as specified by the Competent Authority, and implemented by the Applicant. These may include adaptation of the monitoring plan, the establishment and/or adaptation of risk management measures, or the initiation of new investigations or more in depth studies (in the case where follow-up studies are needed, how they should be designed and who should be responsible for their implementation should be decided by the Competent Authority, in accordance with the monitoring provisions adopted by the Party of Import).
- The Applicant should indicate how monitoring reports could be made available on a central, openly accessible storage and presentation interface (e.g. a publically available website, housed by the Competent Authority) so that it may be more broadly disseminated (including for public awareness and participation). Raw data should be stored by the Applicant and made available for independent review of the data, its interpretation, and conclusions drawn from the monitoring activities. Reporting should also be disseminated, as determined in the monitoring plan, via GMO registers established by the Competent Authority and other public databases.
- The Applicant should submit required information on the social utility of MON89034 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

Overall recommendation

From our analysis, we find that the deficiencies in the dossier do not support claims of safe use, social utility and contribution to sustainable development of MON87427 x MON89034 x NK603 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 **MON87427 x MON89034 x NK603 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 TECHNICAL DOSSIER RELATED TO EFSA/GMO/BE/2013/117

About the event

MON87427 x MON89034 x NK603 maize was produced by crossing parental lines MON87427 and MON89034 x NK603 by conventional breeding methods.

Each of the parental lines were developed through genetic modification.

This stacked event produces the proteins Cry1A.105 and Cry2Ab2.to provide insect tolerance. Cry1A.105 contains elements with a high degree of homology to Cry1Ab, Cry1F and Cry1Ac. The proteins provide protection from feeding damage caused by certain lepidopteran pests.

The event also produces and CP4 EPSPS and CP4 EPSPS L214P proteins to provide tolerance to glyphosate.

This application is for food, feed, processing and import. Application for full range use have been made to Canada and the U.S. Regulatory submissions is also made to countries importing significant amounts of maize (countries like Colombia, Japan, Korea, Mexico, Phillipines and Taiwan). MON87427 x MON89034 x NK603 is not approved for any application in a third country.
The parental lines MON89034 and NK603 are approved for food, feed, import and processing in the EU. Parental line MON87427 is not approved any of these applications in the EU.

MON87427 x MON89034 x NK603 maize has been field tested in the U.S in 2010 at five field sites.

Assessment findings

Safety of Cry genes

MON87427 x MON89034 x NK603 maize combines different classes of Bt proteins named Cry toxins, namely Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1 and Cry34/35Ab1. 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).

Two meta-analyses of published studies on non-target effects of Bt-proteins in insects, (Lövei and Arpaia 2005) in relation to non-target and environmental effects, 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 (Lövei et al 2009). 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, a stack containing Cry1A.105 and Cry2Ab2 is present, together with another insert (CP4 EPSPS).

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.

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 al2015). 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 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., 2014, 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, 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 also often combined with multiple Cry proteins, that could have additive or even synergistic effects on non-target species and the environment.

This stacked GM maize is the result of combining the already glyphosate tolerant stack **MON89034xNK603** with another glyphosate tolerant GM maize**, MON87427**.

Thought the application in question does not encompass the cultivation of **MON87427xMON89034xNK603**, 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.

Since the purpose of the *cp4 epsps* gene cassette (infers glyphosate tolerance) is to be able to treat the maize crop with glyphosate-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 herbicides in the comparative and toxicological assessment of GM crops with herbicidal co-technology.

Glyphosate
In both MON87427 and MON89034xNK603 (where NK603) was the original event with glyphosate tolerance) the glyphosate tolerance is inferred through the presence of genes coding for the CP4 EPSPS protein. Though the genes are slightly different, the resulting CP4 EPSPS proteins are claimed by the applicant to be structurally and functionally equivalent. The presence of two gene cassettes producing CP4 EPSPS is shown in the dossier to additively increase the presence of the CP4 EPSPS protein (table 12, p.79). One may assume that this increase in CP4 EPSPS levels increases the plants tolerance to glyphosate (i.e. the crop can be sprayed more intensely). However, the dossier contains no information concerning the effect on tolerance. Increasing the plants tolerance level might be an attempt to combat the increasing level of glyphosate tolerance in weeds, meaning that higher doses and more repeated applications during the growing season can be used. Glyphosate has long been promoted as an ideal herbicide with low toxicity and little environmental impact (Duke and Powles, 2008, Giesy et al., 2000). H owever, in recent years glyphosate has received a lot of risk-related attention. This is partly due its increased use since the introduction of glyphosate-tolerant GM-plants (Dill et al., 2010, Cuhra et al., 2013), and reports on negative effects in terrestrial and aquatic ecosystems (Blackburn and Boutin, 2003, Solomon and Thompson, 2003). In addition, studies on animals and cell cultures indicate that there might be health implications from exposure to glyphosate (Axelrad et al., 2003, Benachour et al., 2007, Cuhra et al., 2013). Among the health effects observed in animal models are histopathological changes in organs such as the liver, cell-division dysfunction in early embryos, negative impact on nerve-cell differentiation, increased fetal mortality, growth reduction, and skeletal malformation. 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).

Recommendation:

- The applicant should include a full evaluation of the co-technology intended to be used with MON87427xMON89034xNK603, namely glyphosate-based herbicide. 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 *cp4 epsps* are outcrossed. High doses and continuous use of a few herbicides promotes development of resistance in weed species, creating a snowball effect where doses used accelerate in response to weed resistance evolution. The herbicide will never be confined to the field but will also affect surrounding areas/ecosystems such as forests, meadows and aquatic run-off systems.

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 argue 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 relatives, and on the herbicide application regime is essential for evaluating the sustainability and environmental impact of this crop. Thus, we would like the ERA to consider the risks connected also to the cultivation of the crop.

Recommendation:

- The regulators are encouraged to ask the Applicant to provide a full ERA of the life cycle of MON87427xMON89034xNK603 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 (even when the application does not include cultivation).

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.

Many parts of this safety assessment is derived from previous finding with the parental lines MON87427 (EFSA/GMO/BE/2012/110 Monsanto Company) and MON89034 x NK603 (EFSA/GMO/NL/2009/72 (Monsanto Company/ Dow Agro Sciences LLC). In general, the applicant describes most of the traits and characteristics of the "stacked event" as being the same as those of the parental GM events used in production of GM maize. The applicant has not demonstrated that interactions among the different transgenic proteins, particularly for allergenic or toxic effects, are not taking place in this event, despite evidence of potential effects for Cry proteins described. Assumptions-based reasoning based on parental lines 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 on the parental lines or single events.

MON87427 x MON89034 x NK603 maize combines Bt proteins active against insects pest (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.

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.

Identification and characterization

2. Molecular characterization:

Information on the sequences actually inserted/deleted or altered:
Southern Blotting:
Southern blotting was employed by the applicant to confirm the presence of the inserts within the stacked event, and demonstrate their stability after having undergone cross-breeding to generate this event.
"*The migration of the bands in Figure 8 and Figure 9 are slightly altered when compared to the migration of the molecular weight markers and between different DNA preparations. These altered migrations are likely the result of a difference in salt concentrations between the genomic DNA samples and the molecular weight marker*."

- Figures 8 and 9 are missing, or the text description does not match the images provided.

- The absence of molecular weight markers on any of the six images (figures 4 - 6) is problematic since the molecular weight marker allows the size of the detected bands to be gauged, and it is important for interpreting the results of such an experiment.

- Longer exposure times for some Southern blots, such as those of figure 5, are recommended since bands reported by the applicant are hardly visible on the images. While we acknowledge that longer incubation times will render the more prominent bands darker, being able to see the fainter bands is more important. We encourage the applicant to supply both images (i.e. the longer and shorter incubation times) so that all bands are clearly visible.

- The applicant makes use of a number of large probes (figure 5 contains one which is approximately 1.5 kb long, for example), which reduces the ability of the Southern blot to detect small deletions, insertions, rearrangements and point mutations, and could thus give false negative results for such occurrences. We recommend that the applicant use smaller probes in order to reduce this risk.

These recommendations have been made assuming that the applicant continues to make use of Southern blots to detect and characterize inserted, deleted or altered sequences. However, it should be pointed out that more sensitive and informative techniques have become available, and we encourage the applicant to consider using them to supplement or replace Southern blots in their applications. A number of profiling techniques have been suggested (Heinemann, Kurenbach & Quist 2011), but we wish to highlight:

- Southern-by-Sequencing (SbS): Harnessing the sequencing power of next generation sequencing (NGS) with in-solution sequence capture techniques, this method was designed to be used in the screening process during the development of GM crops to weed out unsatisfactory transformants. In addition to being able to confirm copy number and intactness of inserts, the authors report that this technique was able to detect single nucleotide polymorphisms, provide detailed information about the flanking

sequences of the insert, and detect fragment insertion which fall outside of the coverage of primers and probes used in PCR and traditional Southern blotting (Zastrow-Hayes et al. 2015).

- Whole genome sequencing: Another application of high-throughput NGS, whole genome sequencing would provide detailed information about the milieu into which the transgenes have been inserted, including information regarding interrupted endogenous genes, small fragment inserts at other loci, and proximity of transposons (Schnable et al. 2009, Zastrow-Hayes et al. 2015).

Organization and sequence of the inserted genetic material at each insertion site:
The Applicant should provide all the primer sequences that were used for on the sequencing studies; the electropherograms should be provided as well in order to check the quality of the sequences; generational sequencing studies should have been conducted.

2.2.3 Information on the expression of the inserted/modified sequences (p.63)

Expression levels of Cry1A.105, Cry2Ab2 and CP4 EPSPS were analysed using ELISA on samples from maize tissue (grain and forage). Plants (whole stack and each single event) were glyphosate treated prior to analysis.

Levels of all Cry proteins were as expected and claimed not to raise safety concerns. Levels of CP4 EPSPS were as expected in the combined expression in MON87427 and MON89034 x NK603.

Bioinformatic analysis of 3' and 5' flanking regions has been made for each of the single events constituting MON87427 x MON89034 x NK603 maize. Peptides of concern were not revealed in this region and no homology to known allergens or toxins were revealed. It is however not clearly stated if it was regions in the stack or the single events constituting itthat were analysed (p. 58). We suggest that the Applicant do so in order to verify potential changes in sequence do to the load of new transgenes being combined and expressed.

3. Comparative assessment (p. 64-65)
The stacked maize event MON87427 x MON89034 x NK603 was compared to a conventional maize variety with a similar genetic background and other commercially available maize hybrids.
Key nutrients and components were selected for analysis based on guidance provided by OECD for maize.

No difference was found for proteins analysed in the stack and the near isogenic line (control). However, two of the 18 aminoacids were found to be significantly different from those in the control (Arginine and Glycine). The Applicant claim that this difference has no relevance to the food and feed perspective without analyzing it further.

For MON 87427 × MON 89034 × NK603 (NT) a total of 59 of the 65 components assessed

were found to be equivalent (Equivalence Category I) or equivalent more likely than not (Equivalence Category II) to the set of commercial conventional reference hybrids at the 95% confidence level. One component (carbohydrates by calculation in grain) was categorized as non-equivalent more likely than not (Equivalence Category III) and another component (calcium) was categorized as non-equivalent (Equivalence Category IV). For the remaining four components (vitamin B2 and sodium in grain and NDF and total fat in forage) equivalence limits could not be well established due to a lack of observable variation in the values for the commercial conventional reference hybrids.

We do not have enough competence to exploit this further, but it is important that it is highlighted and we think it should be followed up further for potential implications that not are based on assumptions.

4. Toxicological assessment

The stacked event produces Cry1A.105, Cry2Ab2 and CP4 EPSPS in the same plant. The potential toxicity of each introduced protein is performed by comparison to known toxins (biochemical characteristics). Toxicity is evaluated based on 1) demonstrated history of safe use, 2) similarity to known toxins or other biologically active proteins, 3) acute toxic effect in mammals and 4) rapidity of digestion in the mammalian gastrointestinal tract.

According to the Applicant, all the proteins have been proven to be safe in previous Applications by EFSA.

All proteins are claimed to be non-toxic based on the four points above. The analysis is based on previous assessments made from the parental lines. It is important that the proteins from the stack in questions are analysed as they are expressed in a different context than in the single parental stacks. This can potentially have an impact on the proteins expressed in the stack and thus their behavior.

An updated bioinformatics analysis was performed on all proteins in this stack. It is however not clear whether these proteins are from the stack itself or bacterially derived.

A bacterial version of the CP4 EPSPS and Cry proteins were used for heat treatment analysis (stability). It must be a goal to use plant derived versions of proteins to be analyzed.
The proteins analysed denatured as predicted upon elevated temperatures. However, the proteins were not analysed in the combination they are expressed in (at the same time) for denaturation effects.

These proteins were also rapidly degraded in simulated gastric fluid analysis. These data were also from previous assessment of parental lines.

Acute oral toxicity studies have not been performed of the proteins from the stack in combination, but on the parental lines and single events.

Repeated dose toxicity studies (28 days, oral, rodent) have not been performed due to the “demonstrated safety of these proteins”. It must be emphasized that the studies performed on

toxicity has been performed on the parental lines only, and not on the stack in the present application. Thus, it is not possible to conclude about potential toxicity from the combination of proteins in this stack expressed at the same time.
We therefore suggest that the Applicant performes toxicity studies with plant derived proteins from the whole stack the Applicant applies authorization for here.
A whole food/feed assessment using a 90 day feeding study was also not considered necessary due to the claimed safety of the parental lines and proteins expressed in these.

Recommendation:

- We suggest that the Applicant perform toxicity studies with plant derived proteins from the stack the Applicant applies authorization for here.

5. Allergenicity assessment

Based on studies performed in parental lines MON87427 and MON89034 x NK603 it is claimed that there is no allergenic risk to humans or animals of CP4 EPSPS, Cry1A.105 and Cry2Ab2. The proteins from the parental lines have been assessed and found not to be alleregenic as 1) they are not from an allergenic source, 2) the protein is present in a small amount, 3) there is no structural (aminoacid) similarities to known allergens, and 4) the proteins are rapidly degraded by pepsin and digestive enzymes.

The proteins have not been assessed in the combination present in the plant during specific serum screening as they as single proteins have been assessed as safe.

Recommendation:

- We encourage the Applicant to analyse proteins isolated from the stacked event to investigate proteins as they are expressed in the plant, and not base safety assessments on data from single events and stacks where proteins are expressed in another context.

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. MON87427xMON89034xNK603 is not yet approved for cultivation in any third country.

As already stated, the Applicant does not provide any data relevant for an ERA of MON87427xMON89034xNK603 (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

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 is not provided by the Applicant.

The MON87427xMON89034xNK603 maize confers tolerance to glyphosate. Recent studies have shown negative effects from glyphosate, both on species present in terrestrial and aquatic ecosystems and on animals and cell cultures (for further elaboration and references on this issue see section p.9-12). Consequently, glyphosate is now increasing recognized as more toxic to the environment and human health than what it was initially considered to be. This is particularly a concern as the introduction of glyphosate tolerant GM plants has led to an increase in the use of glyphosate (Dill et al 2010). As MON87427xMON89034xNK603 is genetically modified to possess two *cp4 epsps* genes (providing glyphosate tolerance), it is likely to assume that this GM maize is tolerant to higher doses of glyphosate. This could further increase the use of glyphosate. Moreover, studies has shown increased levels of herbicide residues in herbicide tolerant GM crops (Bøhn et al. 2014), which could have health impacts on humans and animals

consuming food/feed based on ingredients from this type of GM plants. Finally, weed resistance to glycines in maize cultivation has been vastly documented[1]. The Applicant has not provided information on the contribution of the MON87427xMON89034xNK603 maize to the emergence of glyphosate 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

MON87427xMON89034xNK603 maize does also confer resistance to certain lepidopteran and coleopteran pests. 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

The applicant highlights that the appearance of "volunteer" maize in rotational fields following the maize crop from the previous year is rare under European conditions. Still, an evaluation of the occurrence of volunteer plants in the producing countries and suggested control strategies is important for a sustainability assessment. As stated by the Applicant (part 2.1, page 133): "*Survival of maize is dependent upon temperature, seed moisture, genotype, husk protection and stage of development (….)Volunteers are killed by frost or easily controlled by current agronomic practices, including cultivation and the use of selective herbicides."* 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.

Assessment of alternatives

It is also important to evaluate whether alternative options (e.g. the parental non-GM version of this MON87427xMON89034xNK603 maize) may achieve the same outcomes in a safer and ethically justified way. Furthermore, in order to evaluate whether MON87427xMON89034xNK603 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.

Recommendations:

- 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 MON87427xMON89034xNK603 and its contribution to sustainable development. The information provided by the Applicant must be relevant for the agricultural

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

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 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 MON87427xMON89034xNK603 maize and does therefore not provide sufficient information as required by the NGTA.

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