Vår ref:2017/H_134
Deres ref: 2017/1017

Høringsuttalelse av søknad om markedsføring av genmodifisert mais MON87427 x MON87460 x MON89034 x MIR162 x NK603

EFSA/GMO/NL/2016/134

Under EU forordning 1829/2003

Sendt til

Miljødirektoratet

av

GenØk-Senter for biosikkerhet
Mars 2017

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Vår ref:2017/H_134
Deres ref: 2017/1017

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 22.03.2017

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på offentlig høring av søknad **EFSA/GMO/NL/2016/134**, genmodifisert, stablet maislinje MON87427 x MON87460 x MON89034 x MIR162 x NK603, fra Monsanto Europe S.A/N. V, under EU forordning 1829/2003. Søknaden gjelder bruksområdene mat, fòr, import og prosessering.

Vennligst ta kontakt hvis det er noen spørsmål.

Med vennlig hilsen,

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Vår ref:2017/H_134
Deres ref: 2017/1017

Høringsuttalelse – genmodifisert, stablet mais linje MON87427 x MON87460 x MON89034 x MIR162 x NK603, EFSA/GMO/NL/2016/134, under EU forordning 1829/2003.

Søknad EFSA/GMO/NL/2016/134 omhandler genmodifisert, stablet maislinje til bruksområdene mat, for, import og prosessering.

Den genmodifiserte maisen har toleranse mot herbicider som inneholder glyfosat via de innsatte genene *cp4 epsps* og *cp4 epsps l214p.*

I tillegg er maisen resistent mot insekter av typen Lepidoptera gjennom utrykk av proteiner fra genene *cry1A.105*, *cry2Ab2* og *Vip3Aa20.*

Denne stablete maislinjen utrykker antibiotikaresistens genet *nptII* (Kanamycin og Neomycin resistens), et gen som gir økt avlingsutbytte ved vannmangel (*cspB*), samt genet *pmi* som gjør at de transformerte plantene kan bruke mannose som karbonkilde.

Hverken den stablete maisen eller dens foreldrelingjer er godkjent for noen av bruksområdene i Norge.

I EU er samtlige foreldrelinjer godkjente for de omsøkte bruksområder, men ikke den stablete maislinjen denne søknaden omhandler.

Den genmodifiserte, stablete maislinjen dyrkes i Canada.

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OPPSUMMERING

GenØk–Senter for biosikkerhet, viser til høring av søknad EFSA/GMO/NL/2016/134 om MON87427 x MON87460 x MON89034 x MIR162 x NK603 mais som omfatter bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra denne maisen.

Vi har gjennomgått de dokumenter som vi har fått tilgjengelig, og nevner spesielt følgende punkter vedrørende søknaden:

- Genmodifisert, stablet mais linje MON87427 x MON87460 x MON89034 x MIR162 x NK603 er ikke godkjent i Norge eller EU for noen av de omsøkte bruksområdene.
- MON87427 x MON87460 x MON89034 x MIR162 x NK603 er tolerant mot sprøytemidler som inneholder glyfosat. Dette er sprøytemidler med økt fokus iht potensiell helse og miljøfare ved bruk..
- MON87427 x MON87460 x MON89034 x MIR162 x NK603 inneholder antibiotikaresistens genet *nptII* og er dermed ikke lovlig omsatt i Norge iht matloven (i mat og fôr).
- Søknaden om mais linje MON87427 x MON87460 x MON89034 x MIR162 x NK603 mangler data og informasjon som er relevant for å kunne vurdere kriterier rundt etisk forsvarlighet, samfunnsnytte og bærekraft.

SUMMARY

GenØk-Centre for biosafety refers to the application EFSA/GMO/NL/2016/134 on MON87427 x MON87460 x MON89034 x MIR162 x NK603 maize for import, processing, food and feed or ingredients thereof.

We have assessed the documents available, and highlights in particular the following points for the current application:

- The gene modified, stacked maize event MON87427 x MON87460 x MON89034 x MIR162 x NK603 is not approved for any application in Norway or the EU.
- Maize event MON87427 x MON87460 x MON89034 x MIR162 x NK603 is tolerant to herbicides containing glyphosate. This is a herbicide with increased interest regarding potential health and environmental damage.
- MON87427 x MON87460 x MON89034 x MIR162 x NK603 contains antibiotic resistance marker genes (*nptII*) and are thus banned in Norway through the Food Act (in food and feed).
- The application on maize event MON87427 x MON87460 x MON89034 x MIR162 x NK603 lacks data and information relevant for assessment of criteria on ethically justifiability, social utility and sustainability.

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Application on EFSA/GMO/NL/2016/134

The stacked maize event Mon87427 x MON87460 x MON89034 x MIR162 x NK603 contains several distinct classes of genes providing herbicide tolerance *(cp4 epsps* and *cp4 epspsl214p*), insect resistance (*cry1A.105, cry2Ab2* and *Vip3Aa20*), tolerance to less water supply (*cspB*) and selection markers (*nptII* and *pmi).*

Previous evaluations

The Norwegian Scientific Committee for Food Safety (VKM) has commented on the application for maize event MON87427 x MON89034 x MIR162 x NK603 (EFSA/GMO/NL/2016/131) (1) with the following issues:

- Low temperatures during processing and concentration of proteins: why are the concentrations of the transgenic proteins reduced and not increased?
- VKM wants an evidence-based statement, rather than a claim made on synergism or antagonism of the transgenic proteins effect on each other. They justify this statement by referring to EFSA guideline (2011) (2).
- There is a need for further clarification on the potential role of the proteins Cry1A.105, Cry2Ab2 and Vip3Aa20 as adjuvants. VKM see this as important in cases where maize is imported for high-protein fractions (as maize gluten meal).
- VKM also refer to the experimental data from the single events and comment that they alone are not sufficient to answer potential uncertainties related to a combined exposure of the transgenic proteins expressed.

VKM evaluated the parental, single event MON87460 (EFSA/GMO/NL/2009/70) in 2010 (3) where they commented, among others, on the following issues:

- Vitamines A and C were not measured and this should be done according to the consensus document.
- Neither the cspB nor the nptII protein is similar to known toxins, and although some parameters were statistically different during a feeding experiment, this was not considered as toxicologically relevant.
- The knowledge on the presence of *nptII* gene in the environment is deficient. Also, issues surrounding potential for increased recombination in exposed bacteria is not fully known.

VKM also evaluated the single, parental event MON89034 (EFSA/GMO/2007/37) in 2014 (4) in a final health and environmental risk assessment with the following comments:

- MON890934 is nutritionally equivalent to conventional maize.
- Proteins Cry1A.105 and Cry2Ab2 are not likely to induce toxic or allergenic reactions
- Environmental risk of MON89034 is comparable to conventional maize with intended usage.

VKM has also evaluated the single, parental event NK603 based on information provided in applications EFSA/GMO/NL/2005/22 and EFSA/GMO/RX/NK603 in a food and environmental risk assessment (2013) (5)with the following comments:

- Maize event NK603 is nutritionally equivalent to conventional maize varieties, and proteins expressed in NK603 is unlikely to induce toxic or allergenic potential.
- Environmental risk of NK603 is comparable to conventional maize regarding environmental risk in Norway

The Norwegian Biotechnology Advisory Board has assessed 27 applications of genemodified maize events for import, processing, food and feed (6) and commented that these maize events do not contribute to sustainable development, has social utility or are ethically justifiable, due to, among others:

- Weed developing resistance against the herbicides faster than with alternative production means/methods,
- The use of herbicides seems to increase in the big GMO producing countries,
- Herbicides containing gluphosinate is documented to be hazardous to health and environment,
- The insecticides produced by the insectresistant plants can harm non-target organisms,
- Pests can develop resistance faster than with alternative production means/methods.

The EFSA panel has evaluated all the parental, single events of maize, and as for event NK603 (7), they all have been evaluated to be as safe as conventional maize, based on the data provided by the Applicant.

GenØk- centre for biosafety has assessed the parental, single events or combinations of these in several occasions previously (http://genok.no/radgiving/horingsuttalelser/).

Of these, GenØk- centre for biosafety has previously assessed a stack containing all parental events except MON87460 in 2016 (8), where we commented on the following:

- The Applicant is encouraged to consider the complexity of a stacked transgenic plant in comparison to single events and evaluate potential safety issues based on that
- We encourage the Applicant to specify more clearly, if the proteins characterized are from the stack or if the statements are based on previous analysis on single events.
- We strongly encourage the Applicant to analyze the proteins in the stack for homology to known toxins or anti – nutrients and not make assumptions based on data from analysis of previously assessed single events (constituting the stack in question).
- We encourage the Applicant to clearify if proteins analysed are from the maize stack or if the data are from the previous analysis of the single events only
- We encourage the Applicant to clarify which pH levels stability analysis have been performed at. A broad pH range will better mimic the situation in the gastric system.
- We recommend the Applicant to perform 28 day oral toxicity analysis of the proteins isolated from the multi stack, as no analysis have been performed on the newly expressed proteins, only on proteins isolated from single events in parental lines, in

previous analysis and also that the proteins in the stack has no history of safe use as they are expressed there, as this is a new combination of traits.

- We encourage the Applicant to perform a 90 day feeding study as the combined expression of traits in this multistack might be potentially distinct from each of the traits being expressed alone and also as no safety data is presented from the proteins isolated from the multistacks and the combined expression of these.
- We recommend the Applicant to consider performing the analysis for allergenicity on proteins as they are expressed in the multistack and not base assumptions on data from the single parental events alone.

GenØk assessed the single parental event MON87460 in 2010 (9), where the following comments were made:

- It was not provided enough data to evaluate the scientific quality of the application.
- There are knowledge gaps regarding potential health effects of the protein CspB.
- One should prevent the use of the antibiotic resistence gene *nptII* to avoid transfer to bacteria(s) in the gastrointestinal tract.
- There was not data to evaluate sustainability, social utility or ethical aspects as according to the Norwegian gene technology act (NGTA) (10).

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Social utility and sustainability issues on maize event MON87427 x MON87460 x MON89034 x MIR162 x NK603, EFSA/GMO/NL/2016/134

In addition to the EU regulatory framework for GMO assessment, an impact assessment in Norway follows the Norwegian Gene Technology Act (NGTA) (10). In accordance with the aim of the NGTA, production and use of the GMO needs to be *ethically justifiable*, demonstrate a *benefit to society* and contribute to *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"* (See section 17 and annex 4 for more detail on the regulations on impact assessment). Recent developments within European legislation on GMOs allow Member States to restrict the cultivation of GMOs on their own territory based on socio-economic impacts, environmental or agricultural policy objectives, or with the aim to avoid the unintended presence of GMOs in other products (Directive 2015/412) (11). Additionally, in recent years, attention increased within academic and policy spheres to broaden the assessment of new and emerging (bio) technologies to include issues that reach beyond human and environmental health. (12-17).

With the assessment of *ethically justifiability, benefit to society* and *sustainability* as in the NGTA, significant dedication is demanded as it covers a wide range of aspects that need to be investigated (e.g. Annex 4 within the NGTA, or 18). Nevertheless, the applicant has currently not provided any information relevant to enable an assessment of these criteria. Therefore, this section will highlight some areas that are particularly relevant to consider with maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 and where the applicant should provide data for in order to conduct a thorough assessment according to the NGTA.

Sustainability

The maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 contains particular events that confer tolerance to herbicides that contain 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 on Herbicides), as well as in villages in areas where glyphosate is used systematically as part as the GM crops tolerant to glyphosate (19). Consequently, glyphosate is now increasingly 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 (20, 21). As maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 is genetically modified to possess genes that provide glyphosate tolerance, it is likely to assume that this GM crop is tolerant to higher doses of glyphosate and could potentially further increase the use of glyphosate.

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 (22). Therefore, considerations of the co-products also warrant an evaluation of safe use and the Applicant does not provide data required for such an assessment.

Impacts in producer countries

As already stated, the Applicant does not provide data relevant for an environmental risk assessment of maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 as it is not intended to be cultivated in the EU/Norway. However, this information is necessary in order to assess the sustainability criteria as laid down in the NGTA. This criteria is referring to a global context, including the contribution to sustainable development in the producing countries with a view to the health, environmental and socio-economic effects in other countries, in this case where the maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 is cultivated.

In addition to a lack of information, there can also be ambiguity about how scientific conclusions may be achieved. For example, 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. This is particularly relevant to consider as field trials of the maize have been in the USA, while cultivation will be in Canada and no information about how this difference may affect the risk evaluation has been provided.

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. 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.

Herbicide –resistant genes

When herbicides are used in agriculture, it is important to minimize the potential of weeds becoming resistant. Indeed, when crops are engineered to be herbicide tolerant in order to maintain an agricultural practice that uses herbicide, it is essential to remain attentive to the amount of herbicide used, the potential consequences of this use for the area in which the crop is cultivated and develop management strategies to make sure that this does not create (more) resistant weed. Moreover, studies have shown increased levels of herbicide residues in herbicide tolerant GM crops (e.g. 23), which could have health impacts on humans and animals consuming food/feed based on ingredients from this type of GM plants. The Applicant has not provided information on whether the cultivation of maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 could affect the emergence of glyphosate resistance in weeds, nor if there are cases of this in the areas intended for cultivation of the variety which are also important aspect to evaluate the ethical justifiability. Furthermore, this maize is cultivated in Canada, where glyphosate resistant weeds has increased significantly[1]. Additionally, no information is currently provided by the applicant that demonstrates reflection on how the monitoring, assessment or evaluation of the GM crop in countries where the crop will

[1] http://weedscience.org/Summary/Country.aspx Status of Herbicide Resistance in Canada, Accessed on 15 March 2017.

potentially be cultivated is assessed, as the applicant considers information on this not relevant because maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 will not be cultivated in Europe. However, it remains an important aspect for a sustainability evaluation and thus necessary if the application is to be evaluated according to this criteria in the NGTA.

Drought tolerance

The purpose of the event MON87427 x MON87460 x MON89034 x MIR162 x NK603 is, among others, to achieve drought tolerance. This is an interesting trait with regard to potential for growth under climatic difficult conditions. Hence, the plant may contribute to increased food security when confronted to dry or water limited conditions. However, the applicant does not provide any documentation that supports that the event MON87427 x MON87460 x MON89034 x MIR162 x NK603 maize is effective in producing a viable yield under such conditions, the extent to which the contribution to abiotic stress can be attributed to the genetic modification, and the portion that can be attributed to the base genetic of the host plant. Documentation with regard to yield loss under dry and water limited conditions, particularly during flowering and grain fill periods when maize yield potential is most sensitive to stress, should therefore be provided in order to assess the added value of the recombinant trait and a comprehensive assessment of the sustainability criteria.

In sum, a proper evaluation of potential impacts that are relevant for the sustainability criteria in the producing country is lacking, and sufficient information in this agricultural context needs to be provided. This should 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.

Benefit to society

The criteria of 'benefit to society' in the NGTA should be interpreted on a national level. That means that the import of maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 needs to demonstrate how it will benefit Norway. However, the applicant provides no information on this part. Indeed, the applicant state that this maize will replace maize in existing food and feed products. It is therefore important to evaluate how GM crops in general, GM maize in particular, and the use of GM maize in food and feed are valued by Norwegian consumers. This information will contribute to anticipate impacts at an early stage, as well as that it may demonstrate a need to assess the alternative options for import of maize. However, the limited amount of empirical data available on the attitude of Norwegian citizens towards GM (e.g. 24, 25) is outdated and more empirical research on this is warranted to investigate consumers' attitude, demand and acceptance. Furthermore, 29 % of the global maize production is GM. It is therefore not a problem for Norway to import GM free maize and therefore no need to replace current imports. The GM maize in question does also not contain any beneficial characteristics for consumers that would prioritize this maize over non-GM maize.

Assessing alternatives

When a new (bio-) technology is developed, it is important to reflect on what problem it aims to solve and to investigate whether alternative options may achieve the same outcomes in a safer and ethically justified way. After all, when a crop is genetically modified to tolerate a

particular herbicide, it means that the crop is developed for a particular cultivation practice in which these herbicides are to be used. What is meant with alternatives, and what would benefit from being assessed could include alternative varieties (e.g. non-GM) for import, alternative sources to satisfy the demand, alternative ways of agriculture, or even explore alternative life visions. In fact, this corresponds with the increased trend within research and policy of science and innovation to anticipate impacts, assess alternatives, reveal underlying values, assumptions, norms and beliefs (15, 26) in order to reflect on what kind of society we want, and assess how certain (biotechnological) developments may or may not contribute to shaping a desired future. Thus, in order to evaluate whether maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 contributes to social utility, it is important to investigate current and future demands and acceptance of this in Norway and if there are alternatives sources for maize that could be cultivated elsewhere that may satisfy this demand, or are more desirable.

Ethical considerations: socio-economic impacts

As known, GM crops have been, and still are, a hot topic for debate. A significant amount of this debate focuses on the safety of GMOs and currently no scientific consensus on this topic has been achieved (27). Nevertheless, another substantial part of the debate is around the socio-economic impacts of GM productions and many questions for evaluating the above mentioned criteria in the NGTA are based on an assessment of the socio-economic impacts. These impacts can vary and range from seed choice for farmers, co-existence of different agricultural practices, changing power dynamics among stakeholders, new dependencies of farmers, intellectual property right on seeds, benefit sharing, the decreasing space for regional and local policy, and more organisational work and higher costs for non-GM farmers (e.g. for cleaning of sowing machines or transport equipment to avoid contamination). Although the examples of socio-economic impacts clearly indicate the complexity and extensive list of concerns beyond safety aspects, little empirical investigation on these kind of aspects has been done. For example a study performed by Fischer et al. (28) concerning social implications from cultivating GM crops found that from 2004 – 2015 there has only been 15 studies corning socio-economic implications of cultivating Bt-maize. The study demonstrates that published literature is dominated by studies of economic impact and conclude that very few studies take a comprehensive view of social impacts associated with GM crops in agriculture. The amount of research performed in this case and the minimal focus on social impacts strongly indicate a high need for further investigation on how the cultivation of GM crops affects different parties involved. It is therefore striking that no information on any of the above mentioned points is discussed by the applicant.

Co-existence

The cultivation of GM plants in general is causing problems with regard to co-existence, an important socio-economic impact. For instance, Binimelis (29) has investigated consequences on co-existence of Bt maize in Spain among small-scale farmer and has found that co-existence is very difficult and that farmers in some areas have given up growing non-GM maize. Even though the cultivation of maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 is not planned in Europe/Norway, it is important to obtain information about the strategies adopted to ensure co-existence with conventional and organic maize production and information about consequences on co-existence in the countries intended for production of

maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 and minimize the likelihood for gene flow to wild relatives. Legal information could inform assessing organs on the governance strategies and possibilities to ensure co-existence, although it has been noted that this may not suffice, as co-existence has become an arena of opposed values and future vision of agriculture and the role of GM crops within these visions (30). Indeed, although a framework for maintaining co-existence in Europe was established in 2003 (31) this effectively meant technical measurements and recommendations (e.g. cleaning of sowing machines and transport vehicles) and remains challenging in practice (32, 33). Moreover, this framework arguably reduced the significance of the issue of co-existence to questions concerning economic aspects for individuals (e.g. farmers), rather than recognizing that agricultural practices are interwoven in dynamic social, economic and political systems (34, 35). For the criteria in the NGTA, information on co-existence is required to enable a coherent analysis.

Summary

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 criteria of ethically justifiability, benefit to society and sustainability assessment. An important part that is lacking is information about the consequences of the cultivation of maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 for the producing country. The information provided by the Applicant must be relevant for the specific agricultural context of this country and should also stress the need for information on integrated weed management strategies in those countries (36). Moreover, the information should contain issues such as: Changes in herbicide use, development of herbicide resistant weed, potential for gene flow and possible socio-economic impacts such as poor and/or small-scale farmers in producing countries, share of the benefits among sectors of the society and as explained, effects on co-existence of different agricultural systems. Additionally, the applicant does not attempt to demonstrate a benefit to the community or any reference on the consumer attitude and demand within Norway for maize MON 87427 x MON 87460 x MON 89034 x MIR162 x NK603 and does therefore not provide sufficient information as required by the NGTA.

Environmental risk issues of MON87427 x MON87460 x MON89034 x MIR162 x NK603 maize in a Norwegian context

The level of maize production is very low in Norway and only some varieties can grow in the southern part due to climate conditions. There are also no wild populations of maize in Norway.

These limitations lead to minimal possibilities for establishment of maize outside agricultural practices. Loss of gene modified maize seed through storage or transport would therefore not involve great risk for spread into the wild or spread of transgenes to wild relatives.

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Molecular characterization, expressed proteins and herbicide use -special issues to consider in the present application

The stacked event MON87427 x MON87460 x MON89034 x MIR162 x NK603 maize contains **eight inserted transgenes** expressing several distinct classes of proteins.

The genes *cp4 epsps* and *cp4 epsps l214p* provides the stacked maize with increased tolerance to glyphosate containing herbicides, while the genes *cry1A.105*, *cry2Ab2* and *Vip3Aa20* provides the plant with resistance towards Lepidoptera insects.
This stack also has a gene providing increased yield under conditions where the water supply is low. This gene is called *cspB.*

Additionally, two selection markers have been added; one for resistance against the antibiotics Kanamycin/Neomycin (the gene *nptII*) and one for alternative carbon source usage, mannose, through the addition of a *pmi* gene.

Stacked events
A stacked, genemodified plant should be regarded as a new event, even if no new modifications have been introduced, as the combination itself in the stack is unique for that event. The gene-cassette combination is new and only minor conclusions could be drawn from the assessment of the parental lines, since unexpected effects (e.g. synergistic effects of the newly introduced proteins) cannot automatically be excluded. Stacked events are in general more complex, and it has been an increased interest in the possible combinatorial and/or synergistic effects that may produce unintended and undesirable changes in the plant – like the potential for up- and down regulation of the plants own genes. Interactions within stacked traits cannot be excluded and whether or not the expressed proteins in the plant can give specific immunological effects or adjuvant effects in mammals has been discussed previously (37, 38).

The stack MON87427 x MON87460 x MON89034 x MIR162 x NK603 maize combines several Bt proteins active against Lepidopteran insects pests. It is well known that synergistic and additive effects both between Bt toxins and other compounds do occur (39). Here, the evidence for changes in activity and specificity of Bt proteins dependent on synergistic interaction and extrinsic features are reviewed and discussed. 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.

Molecular characterization
The stacked maize event MON87427 x MON87460 x MON89034 x MIR162 x NK603 have the following inserted genes:

- The *cp4 epsps* and *cp4 epspsl214p* genes (source: *Agrobacterium tumefaciens)* providing tolerance to glyphosate.
- The *cry1A.105*, *cry2Ab2* and *Vip3Aa20* genes (source: *Bacillus thuringiensis*) providing resistance towards Lepidoptera insects.
- The *cspB* gene (source: *Bacillus subtilis*) providing increased yield when water supply is limited.
- The *nptII* and *pmi* genes (source: *Escerichia coli*) providing antibiotic resistance (kanamycin and neomycin) and the possibility to use mannose as carbon source, as selection markers.

Based on the molecular characterization there is not safety concerns raised on the stacked maize event for any of the inserted genes by the Applicant. This is based on both old data assessments from single, parental events and newer bioinformatics analysis.

We still want to highlight one of the inserted genes in the following session.

NptII gene

The *nptII* gene is the most commonly used ARM gene for plant cell selection (40). Several commercialized GM plants are carriers of this ARM gene (40-42).The presence of ARM gene in GM plants and large-scale release in the environment, or use as food or feed, has raised concerns over the past years regarding possible risks for human health and the environment (43-46). One of the main environmental concerns is that the cultivation of GM plants and its use in food, feed and industrial purposes might provide a source of AR genes that will contribute to the development of new drug-resistant bacteria (40, 47-50). The risk of horizontal gene transfer (HGT) of plant-derived ARM genes to soil or gut bacteria resulting in a reduced antimicrobial treatment of animal and human infectious diseases have been claimed to be very low but cannot be excluded (51-54).

Neomycin and Kanamycin, which were previously listed as “highly important” antimicrobials by WHO have now been included in the “critically important” category (55). This is due to a constant increase of bacteria resistant to various different classes of antibiotics. Treatments of some infections will in the future rely on older (aminoglycosides) antibiotics that are not preferred today because of unfavourable side effects and ADME (absorption, distribution, metabolism and excretion) properties.

Phenotypic resistance to kanamycin in soil bacterial communities is quite common (48, 56-58). However, it is well known that some bacterial species are intrinsically resistant to some antibiotics. Concerns emerge over the increasing prevalence of resistance in previously susceptible species and the increased mobility of such traits. At present, there are only a few studies that have been conducted to determine the prevalence of specific ARMG in bacterial populations in natural environments. The wide distribution of antibiotic resistance genes (ARGs) in general, and the potential threats to the human and animal health arising from horizontal gene transfer highlights the importance of identification and monitoring of the presence and level of antibiotics and AMRG in the environment, as it can function as reservoirs for transferable resistance.).

In 1997 the Norwegian parliament asked the Goverment to prohibit the production and import of GM products that contain genes that confers antibiotic resistance. Thus, in the Norwegian Food Act there is a prohibition against antibiotic resistance marker genes in food and feed. There has been some dispensations given to the fish feed industry for GM events to be present in fish feed, a dispensation that not has been in use and withdrawn[2].

Protein expression and characterization of the newly expressed protein(s)

EPSPS proteins

The *cp4 epsps* and *cp4 epspsl214p* genes and corresponding proteins have previously been risk assessed by EFSA and found unlikely to have any adverse effect on human or animal health or environment as they are supposed to be used in this application.

Cry poteins

MON87427 x MON87460 x MON89034 x MIR 162 x NK603 maize combines two Bt proteins named Cry1A.105 and Cry2Ab2. These proteins, also called Bt-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 (59-62).

Two meta-analyses of published studies on non-target effects of Bt-proteins in insects, (Lövei and Arpaia (63) 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 (64). A review by Hilbeck and Schmidt (62) on Bt-plants, found 50% of the studies documenting negative effects on tested invertebrates.

Additionally, a review by van Frankenhuyzen (65) 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. 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 (66).

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 (67). 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,

[2]https://www.mattilsynet.no/planter_og_dyrking/genmodifisering/bakgrunn_for_avslag_om_aa_bruke_genmodifisert_fiskefor.16613

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. Research on aquatic environments with emphasis on the impact of Bt-crops on aquatic invertebrates including *Daphnia magna* (59)and caddisflies (68)has also been performed. 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 (69)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 (70). Impacts on soil microflora and fauna, including earthworms (71), mychorizzal fungi (72)and microarthropods in response to Cry endotoxins have also been reported (73-75). The significance of tri-trophic effects of accumulation, particularly of insecticidal Cry toxins (76, 77) 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 (78), 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 Baxter et (79) 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. Bravo and Soberón (80) commented on this effect, acknowledging that gene stacking is not a universal solution to resistance development towards Cry proteins. Studies such as these ask 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 relation to health impacts, a publication by Dona and Arvanitoyannis (81) 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 (82). Further studies should also include animals with immune-deficiencies and/or animals exposed to other stress agents simultaneously.

Vegetative insecticidal proteins (Vip)
VIP is one of a number of extracellular compounds, in addition to crystal-associated toxin polypetides, that may contribute to the virulence of *B. thuringensis* (83). These proteins have shown to have a broad insecticidal spectrum, which includes activity against a wide variety of lepidopteran as well as coleopteran pests and they may represent a new generation of insecticidal toxins that could be efficacious against insects that are resistant to Cry toxins (84,

85). In that regard, one strategy involves the presentation of several toxins together, especially if a differing mode of action involving different receptors is available (86).

In this stack, there are two Cry proteins and one VIP protein. The VIP and Cry proteins seem to have the same target species. Although the VIPs may have different mode of action dependent on the target (87). However, special concern or vigilance should be paid to GM stacks that combine events that have similar type of mode of action through their expressed transgenic proteins. Also, the Cry proteins can attach to the same receptor, changing their mode of action. In theory, the presence of two toxins can result in cross resistance and a changed effect on target and also non-target species (87-90). Especially, an overall toxicity study of the GM stacked event should have been considered. For the VIP proteins, MIR 162 has previously been assessed expressing the Vip3Aa20 protein. Previous evaluations of this protein have especially noted the potential cross binding to receptors in the epithelial cells of the gut between Cry and VIP proteins. As this receptor has not been characterised, the similarity to human gut receptors cannot be clarified and should thus be further analysed. This is however not mentioned in this application as potential.

Cold shock protein B (CspB)

Abiotic stresses such as drought, salt, heat and cold are major environmental factors that affect plant growth and development. The drought-tolerant maize MON 87460, which is a parental line in the stack MON87427 x MON87460 x MON89034 x MIR162 x NK603, provides a yield benefit when yield is limited by water availability: (91-93).

As part of the safety assessment of GM crops, the CspB protein has been subjected to the tests required and evaluated according to the criteria on history of safe use, bioinformatics comparing similarity to known toxins and allergens, stability to digestive enzymes, acute toxicity tests a.s.o. The proteins has been evaluated to be safe.

Phosphomannose isomerase (Pmi)

Phosphomannose isomerase (Pmi) is an enzyme that was inserted as a selection marker in parental event MIR162. It allows a positive selection for transformed plants having this gene expressed due to the ability of metabolizing mannose. EFSA evaluated this protein in the single parental event MIR162 (94) to have no safety concerns.

Summary:

- *The nptII and pmi genes are used as a selection markers.*
- *There is a prohibition against antibiotic resistance marker genes through the Food Act.*
- *Safety assessments are mainly based on data from assessments of the single, parental events of MON87427 x MON87460 x MON89034 x MIR162 x NK603*

Herbicides

The maize event MON87427 x MON87460 x MON89034 x MIR162 x NK603 contains cp4 epsps and cp4 epspsl214p genes providing glyphosate tolerance.

Herbicide use on GM plants

Herbicide tolerant (HT) plants are sprayed with the actual herbicide(s), leaving the weed to die whereas the plant with the inserted genes will survive. However, the issue on accumulation of herbicides in the HT plants, including metabolic pathways and metabolites of these, are often not tested as part of the risk assessment of HT plants. Bøhn et al. (95) 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 have the potential for negatively to affect the feed quality of HT GM soybeans (96, 97). It is important to look at the potential metabolites of the herbicides in use and if these are documented to have a negative effect on health and environment.

Glyphosate tolerance

The *cp4 epsps* and *cp4 epspsl214p* genes present in MON87427 x MON87460 x MON89034 x MIR162 x NK603 maize confers tolerance to herbicide products containing glyphosate.

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

Glyphosate has been announced as an ideal herbicide with low toxicity for operators, consumers and the environment surrounding agriculture fields (36, 98). However, it has received more risk-related attention due to its potential for negative effects on both aquatic and terrestrial ecosystems (99), as well as in studies in animals and cell cultures that have indicated possible negative health effects in rodents, fish and humans (100-102).

Studies indicate that agriculture of GM plants is associated with greater overall usage of pesticides than the conventional agriculture (103).

A restricted number of publications indicate unwanted effects of glyphosate on health (102, 104), aquatic (105) and terrestric (99, 106) organisms and ecosystems.

A study of Roundup effects on the first cell divisions of sea urchins (107) is of particular interest to human health. The experiments demonstrated cell division dysfunctions at the level of CDK1/Cyclin B activation. Considering the universality among species of the CDK1/Cyclin B cell regulator, these results question the safety of glyphosate and Roundup on human health. In another study (100) it was demonstrated a negative effect of glyphosate, as well as a number of other organophosphate pesticides, on nerve-cell differentiation. Surprisingly, in human placental cells, Roundup is always more toxic than its active ingredient. The effects of glyphosate and Roundup were tested at lower non-toxic concentrations on aromatase, the

enzyme responsible for estrogen synthesis (108). The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation. The authors conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. They suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.

Additionally, the International Agency for Reseach on caner (IARC) released a report concluding that glyphosate is "probably carcinogenic to humans"(109).

Summary:

- Maize event MON87427 x MON87460 x MON89034 x MIR162 x NK603 is tolerant to glyphosate.
- Potential for accumulation of the herbicides should be considered in GM plants used in food and feed.

Allergenicity and toxicity issues

The EPSPS protein has been evaluated by EFSA in several applications previously and considered to be safe.

The maize event MON87427 x MON87460 x MON89034 x MIR162 x NK603 express two EPSPS proteins, from the genes *cp4 epsps* and *cp4 epspsl214p*.

Toxicological assessment

All toxicological assessments of each novel protein expressed in the stacked maize MON87427 x MON87460 x MON89034 x MIR162 x NK603 are based on the data from previous applications of single parental events of subcombinations of these (p. 49, Technical dossier). These assessments are based om the criteria of biochemical characterizations, bioinformatics, stability under relevant storage and processing conditions, resistance to proteolytic enzymes a.o.

A whole food feeding study was found unnecessary based om previous assessments of the single proteins, but also according to EFSA (110) stating that this is not needed if it already has been demonstrate that it is not biologically different from their conventional counterparts by molecular, compositional, phenotypic and agronomic analysis.

Allergenicity

Due to expected biochemical characteristics of the proteins, assessments of the proteins in previous applications, and their equivalence to corresponding proteins produced in microbial hosts showing that molecular weight, glycosylation pattern, amino acid sequences and enzyme activity are comparable, the applicant has no concern regarding allergenicity of the proteins expressed in the maize event MON87427 x MON87460 x MON89034 x MIR162 x NK603. In addition, the proteins are rapidly degraded in simulated gastric fluids and inactivated by heating,

criteria that are used to assess allergenicity of proteins. The bioinformatics studies that are performed also show that there is no similarity to known toxins or allergens present. A specific serum screening is thus not necessary according to the Applicant.

Adjuvancy effects

In the adjuvancy evaluation (p.57 in Technical dossier), there is no mention of Cry proteins as potential adjuvants. However, the potential adjuvancy of Cry proteins has previously been addressed by the GMO Panel of the Norwegian Scientific Committee for Food Safety (111). Also, scientific studies have shown that the Cry1Ac protein is a potent systemic and mucosal adjuvant (112). 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" (Norwegian Scientific comitee for Food Safety (2013), Evaluation of EFSA/GMO/UK/2007/48).*

We also agree with these concerns and highlight them for the present stack of maize and that this potentially also might be the case for the proteins expressed in MON87427 x xMON87460x MON89034 x MIR162 x NK603 maize.

Summary:

- Toxicology and allergenicity assessment of proteins expressed in this stack is based on data from previous assessments, except newer bioinformatic studies.
- There is a potential for non-target effects by cry proteins that needs to be addressed, 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,a s well as the potential for cry proteins as adjuvants..

Potential interactions between newly expressed proteins

Mode of action, molecular analysis of the corresponding genes and activity of proteins of maize event MON87427 x xMON87460x MON89034 x MIR162 x NK603 made the basis for the conclusion made by the applicant that there are no indications of potential interactions of safety concern between the traits expressed. However, VKM has previously raised the issue on an evidence based statement on this issue, rather than a claim (1), referring to another maize stack.

Hazard identification
According to the applicant, it is unlikely that the proteins expressed from the gene modified maize event will be hazardous.

Main summary

The stacked maize event MON87427 x xMON87460x MON89034 x MIR162 x NK603 is tolerant to herbicides containing glyphosate that has potential health and environmental dangers upon use. Thus, the issue on accumulation should be considered for GM plants to be used in food and feed.

In addition, following the Food Act of Norway, it is prohibited with antibiotic resistance marker genes that is present., thus the stacked event MON87427 x xMON87460x MON89034 x MIR162 x NK603 contains *nptII*, and should thus be prohibited.
The applicant should provide data relevant for assessment of social utility and sustainable development according to the NGTA(10).

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