Vår ref:2014/H_103
Deres ref: 2014/11536

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 21.10.14

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på høringen av søknad **EFSA/GMO/DE/2011/103** fra Syngenta Crop Protection AG som gjelder mat, fòr, import og prosessering av genmodifisert mais **Bt11xMIR162xMIR604x1507x5307xGA21**.

Vennligst ta kontakt hvis det er noen spørsmål.

Med vennlig hilsen,

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Vår ref:2014/H_103
Deres ref: 2014/11536

Assessment of the technical dossier submitted under EFSA/GMO/DE/2011/103 for approval of Bt11xMIR162xMIR604x1507x5307xGA21 maize

Sent to

Norwegian Environment Agency

by

GenØk- Centre for Biosafety
October 2014

KONKLUSJON PÅ NORSK

Vi trekker frem mangler i dossieret som ikke gir grunnlag for en konklusjon om sikker bruk, samfunnsnytten og bidrag til bærekraftighet av **Bt11xMIR162xMIR604x1507x5307xGA21 mais**. Søker har ikke inkludert noe av den informasjonen omkring samfunnsnytten og bærekraftighet til **Bt11xMIR162xMIR604x1507x5307xGA21 mais** som kreves i den norske genteknologiloven (Appendix 4) for godkjenning i Norge.

Hovedkonklusjon og anbefalinger
Genøk–Senter for Biosikkerhet viser til brev fra Miljødirektoratet angående høring som omfatter **Bt11xMIR162xMIR604x1507x5307xGA21 mais** for bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **Bt11xMIR162xMIR604x1507x5307xGA21 mais**.

Søker gir ikke opplysninger som adresserer vurderingskriteriene bærekraft, samfunnsnytten 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 **Bt11xMIR162xMIR604x1507x5307xGA21 mais** til import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **Bt11xMIR162xMIR604x1507x5307xGA21 mais.**

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av **Bt11xMIR162xMIR604x1507x5307xGA21 mais** til import og prosessering og til bruk i fòr og mat som det søkes om.

SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/DE/2011/103

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 **Bt11xMIR162xMIR604x1507x5307xGA21 maize**, setting out the risk of adverse effects on the environment and health, including other consequences of proposed release under the pertinent Norwegian regulations.

In this application from Syngenta Crop Protection AG, the Applicant is referring to the molecular data used for the genetic modification of each of the single events: Bt 11, MIR 162, MIR604, 1507, 5307 and GA21. We have previously commented on sub-combinations and single events of **Bt11xMIR162xMIR604x1507x5307xGA21 maize** in:

- EFSA/GMO/DE/2010/86 for Bt11xMIR162x1507xGA21 (our previous comments from July 2012: **H_86**)
- EFSA/GMO/DE/2011/95 for 5307 (our previous comments from August 2011: **H_95**)
- EFSA/GMO/UK/2010/83 for MIR604 (our previous comments from March 2011: **H_83**)

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 include long term exposure/feeding studies in a risk assessment before a GM plant product is released on the marked for food/feed consumption.

- The regulator is encouraged to ask the Applicant to consider that we find that it would be ethically incongruous and a double standard of safety for Norway to ban the use of certain herbicides domestically as a health concern, but support its use in other countries.

- 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 data for analysis of potential combinatory effects by the use of appropriate feeding trials.

- The regulator is encouraged to ask the Applicant is recommended to analyze for allergenicity of the proteins in combination using SGF and also serum screening analysis.

- The regulator is encouraged to ask the Applicant to address the Environmental risk assessment interactions between proteins in more detail. Experiments should account for the high total amount of Bt protein in Bt11xMIR162xMIR604x1507x5307xGA21 maize and for possible interactions of the mixture of genes and gene products.

- The regulator is encouraged to ask the Applicant to address the potential for cross resistance between Cry- and VIP proteins and a changed effect on target. Also non-target species should be investigated.

- The regulator is encouraged to ask the Applicant to address the potential of non-target effects of Bt toxins.

- The regulator is encouraged to ask the Applicant to perform a stress test to see how the cyt c and GA21 performs under stress compared to unmodified genetically similar maize.

- The regulator is encouraged to ask the Applicant to survey for Vip3A resistance alleles prior to the use of this toxin.

- The regulator is encouraged to ask the Applicant to supply data on the expression of the novel traits in this stack in combination with the herbicide(s), since this is how it will be grown.

- The regulator is encouraged to ask the Applicant to comment the magnitude of the statistical difference [in expression] when observed, in what kind of tissues these differences are observed, and if it has a potential impact on the actual insect-resistance capability of the plant (stacked event) and potential for development of Cry-resistance in the targeted insects.

- The regulator is encouraged to ask the Applicant to investigate the effect of stressful conditions (such as drought) on the expression levels of the transgenic proteins in different tissues, and to determine whether this may have a potential impact on the actual insect-resistance capability of the plant (stacked event) and potential for development of Cry-resistance in the targeted insects. This should also be done in combination with the trait-specific herbicides which will be used during cultivation.

- The regulator is encouraged to ask the Applicant to comment on whether the lack of equivalence (in several cases equivalence to the reference lines could not be established) could be due to the single genetic modifications (i.e. single events differ from conventional), or if there is an effect of stacking the traits (the stacked events are more different from the conventional than the single events are).

- The regulator is encouraged to ask the Applicant to acknowledge the context of use for the stacked event and its complimentary herbicide technologies and also test for to test for herbicide residues.

- The regulator is encouraged to ask the Applicant to submit required information on the social utility of Bt11xMIR162xMIR604x1507x5307xGA21 maize and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

- The regulator is encouraged to ask the Applicant whether the inclusion of a glyphosate tolerance trait in this event, and implied glyphosate use, can be considered a sustainable weed control solution, given the spread of glyphosate resistant weeds in many cropping systems.

- The regulator is encouraged to ask the Applicant to submit required information on authorized, alternative and sustainable methods to control volunteers in Europe.

Our previous recommendations on sub-combinations and single events of **Bt11xMIR162xMIR604x1507x5307xGA21 maize**:

Application: EFSA/GMO/DE/2010/86 for Bt11xMIR162x1507xGA21 (our previous comments from July 2012: H_86)

- The Applicant should demonstrate the lack of interactive effects between transgenic proteins in this stacked event through proper scientific testing and evidence gathering, rather than justify the lack of testing based on assumptions-based reasoning of no effects.

- Environmental risk assessment interactions between the proteins should be addressed in more detail and experiments should account for the high total amount of Bt protein in Bt11 x MIR162 x 1507 x GA21 maize in each plant part, including pollen, and for possible interactions of the mixture of genes.

- The Applicant should explain the reason for referring to Vip3Aa20 instead of the actual Vip3Aa19 protein in this application, and conduct equivalency testing of the two proteins.

- The regulator is encouraged to address the potential of non-target effects of Bt toxins.

- The regulator is encouraged to consider the safety of co-products intended to be used with the GM event in the evaluation of safety.

- The Applicant should give explanations/clarifications for observed statistically significant differences in the analysis of the proteins expressed in the stack Bt11xMIR162x1507xGA2. What is (are) the biological relevance of these differences?

- The Applicant should provide data showing that the individual proteins from the stacked event have no difference in amino acid sequences from equivalent proteins produced in single events.

- The combined effect of potential allergens in the stack should be investigated.

- Since one of the single events, MIR 162 has not been fully evaluated by EFSA, specific concern or attention should be given to their toxicity assessment.

- The Applicant should submit required information on the social utility of Bt11 x MIR162 x 1507 x GA21 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

Application: EFSA/GMO/DE/2011/95 for 5307 (our previous comments from August 2011: H_95)

- The Applicant should provide information about potential functional and structural changes that may have occurred in the production of the chimeric Cry3A-Cry1AB transgenic protein in 5307.

- The Applicant should provide additional data using a comprehensive set of smaller probes to establish the presence or absence of backbone vector DNA sequences at a limit of detection of ≤ one target/tetraploid genome.

- The Applicant should clarify the functional status of the transgenic protein after processing with properly designed experiments, and further test the effects of 5307 inhalation in animals that are used as models of acute respiratory syndrome, compared with inhalation of the proper conventional comparator. This should include an analysis of allergenicity and toxicity.

- The Applicant should provide data from proper immunostimulation and allergenicity testing of 5307 including tests from diet and inhalation exposures.

- The Applicant should provide experimental data on protein specificity to substantiate claims of equivalence between the test protein and the in-planta produced form.

- The Applicant should submit required information on the social utility of 5307 and its contribution to sustainable development, in accordance with the Norwegian Gene technology Act.

Application: EFSA/GMO/UK/2010/83 for MIR604 (our previous comments from March 2011: H_83)

- The Norwegian Environment Agency should question the value and use of resources in evaluation an application for approval where the Applicant states it does not intend to actually market this event, but rather a similar event with which the target gene is included as part of a stacked event.

- The Norwegian Environment Agency are encouraged to follow up with the outstanding issues raised by member countries in the evaluation of EFSA-GMO-UK-2005-11. Specifically, the Applicant should submit a more detailed plan for post-release monitoring compliant with Annex VII of Directive 2001/18/EC

- The Applicant should submit required information on the social utility of MIT604 and its contribution to sustainable development (including data on pesticides usage and potential benefits of the transgenic trait based on target pest distribution in Norway) 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 **Bt11xMIR162xMIR604x1507x5307xGA21 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.** Hence at minimum, the dossier is deficient in information required under Norwegian law. 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 **Bt11xMIR162xMIR604x1507x5307xGA21 maize**, we conclude that based on the available data supplied by the Applicant, the Applicant has not substantiated claims of environmental safety satisfactorily or provide the required information under Norwegian law to warrant approval in Norway at this time.

ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/DE/2011/103

About the event

The **Bt11xMIR162xMIR604x1507x5307xGA21** maize is GM maize that is produced by conventional breeding crosses of the GM maize events: Bt11, MIR162, MIR604, 1507, 5307 and GA maize. No further genetic modification to produce this stack has taken place. Different vectors were used to produce the single events.

Assessment findings

Herbicides

Glyphosate tolerance

The **Bt11xMIR162xMIR604x1507x5307xGA21 maize** contains the *CP4EPSPS* gene from *Agrobacterium tumefaciens strain ABI* that confers tolerance to herbicides containing glyphosate.

Glyphosate has been heralded as an ideal herbicide with low toxicity for operators, consumers and the environment surrounding agriculture fields (Duke & Powles 2008, Giesy et al 2000), but has received more risk-related attention due to its negative effects on both aquatic and terrestrial ecosystems (Blackburn and Boutin 2003, Ono et al 2002, Solomon and Thompson 2003) and studies in animals and cell cultures indicate possible health effects in rodents, fish and humans (Marc et al 2002, Axelrad et al 2003, Dallegrave et al 2003, Jiraungkoorskul et al 2003, Richard et al 2005, Benachour et al 2007, Gasnier et al 2009)

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. The transgene, cp4 EPSPS, used in genetically modified crops was isolated from an Agrobacterium strain. The whole idea is the combined use of the GM plant and the herbicide. Recent studies indicate that agriculture of GM plants is associated with greater overall usage of pesticides than the conventional agriculture (Benbrook 2009). Large proportions of GM agriculture is glyphosate tolerant crops (GT-cultivars) (James 2010).

A restricted number of recent publications indicate unwanted effects of glyphosate on health (Dallegrave et al 2003, Malatesa M et al 2002), aquatic (Solomon K & Thompson D 2003) and terrestric (Ono MA et al 2002, Blackburn LG & Boutin CE 2003); organisms and ecosystems. Some of these may be considered “early warnings" of potential health and environmental risks, and they should be rapidly followed up to confirm and extend the findings.

Studies in animals and cell cultures point directly to health effects in humans as well as rodents and fish. Female rats fed glyphosate during pregnancy demonstrated increased fetal

mortality and malformations of the skeleton (Dallegrave E et al 2003). Mice fed GE soybean demonstrated significant morphological changes in their liver cells (Malatesta M et al 2002). The data suggested that EPSPS-transgenic soybean intake was influencing liver cell nuclear features in both young and adult mice, but the mechanisms responsible for the alterations could not be identified by the experimental design of these studies. Treatment with glyphosate (Roundup) is an integrated part of the EPSPS-transgenic crop application. Nile Tilapia Oreochromis niloticus) fed sublethal concentrations of Roundup exhibited a number of histopathological changes in various organs (Jiraungkoorskul W et al 2003). A study of Roundup effects on the first cell divisions of sea urchins (Marc J et al 2002) is of particular interest to human health. The experiments demonstrated cell division dysfunctions at the level of CDK1/Cyclin B activation. Considering the universality among species of the CDK1/Cyclin B cell regulator, these results question the safety of glyphosate and Roundup on human health. In another study (Axelrad JC et al 2003) it was demonstrated a negative effect of glyphosate, as well as a number of other organophosphate pesticides, on nerve-cell differentiation. Surprisingly, in human placental cells, Roundup is always more toxic than its active ingredient. The effects of glyphosate and Roundup were tested at lower non-toxic concentrations on aromatase, the enzyme responsible for estrogen synthesis (Richard S et al, 2005). The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation. The authors conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. They suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation. In the highly controversial study by Seralini et al (Seralini et al 2012) the authors concludes that long term exposure of lower levels of complete agricultural glyphosate herbicide formulations, at concentrations below official set safety limits, induce severe hormone-dependent mammary, hepatic and kidney disturbances in rats. In a recently published study by Bohn et al (Bohn et al 2014) the authors recommend to focus also on pesticide residues in major crop plants which may have consequences for human and animal health.

Recommendation:

- Long term exposure/feeding studies should be included in a risk assessment before a GM plant product is released on the marked for food/feed consumption.

Glufosinate-ammonium tolerance

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

The **Bt11xMIR162xMIR604x1507x5307xGA21 maize** is tolerant to the active herbicide ingredient glufosinate-ammonium through the insertion of the *pat* gene. The tolerance to herbicide in general is connected to heavier use, and in most cases biotransformation of the active ingredient to another compound which should also be evaluated for toxicity. Though the mechanism of PAT is described in the dossier, the food and feed safety and toxicology testing, and the references to such tests in other transgene plants, has been done without considering the effects of applying glufosinate to the plants.

Recommendation:

- The regulator is encouraged to ask the Applicant to consider that we find that it would be ethically incongruous and a double standard of safety for Norway to ban the use of these herbicides domestically as a health concern, but support its use in other countries.

Stacked events

Until recently, the dossiers submitted for marked authorization almost only covered single GM 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 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 plant's own genes. The possibility that interactions among the stacked traits may take place cannot be excluded, nor can the possibility that the group of expressed toxins in the plant may give specific immunological effects or adjuvant effects in mammals (Halpin 2005, de Schrijver et al, 2007). Then (2009) reviews and discusses the evidence for changes in activity and specificity of Bt proteins dependent on synergistic interactions with extrinsic features. Such changes may critically influence the bioactivity and hence the potential for unintended effects.

Most of the information submitted in this safety assessment is derived from previous finding with the single lines. 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 the potential effects? (Mesnage et al., 2012). Assumptions-based reasoning with single events should not replace scientific testing of hypotheses regarding interactions. GenØk means that stacked events cannot be approved based on the information on the single events.

Bt11xMIR162xMIR604x1507x5307xGA21 maize combines several classes of Bt proteins active against insects pest like Lepidoptera and Western Corn Rootworm. It is well known that synergistic and additive effects both between Bt toxins and other compounds do occur (Then, 2010). Then (2010) 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 justifying the lack of testing based on assumptions-based reasoning of no effects. Alternatively, if interactions are detected, their documentation and characterization would provide valuable insights for the field of genetic engineering.
- Environmental risk assessment interactions between the proteins should be addressed in more detail and experiments should account for the high total amount of Bt proteins in **Bt11xMIR162xMIR604x1507x5307xGA21** maize and for possible interactions of the mixture of genes.

Vegetative insecticidal proteins (Vip)

VIP is one of a number extracellular compounds, in addition to crystal-associated toxin polypetides, that may contribute to the virulence of *B. thurungensis* (Liu et al 2007). These proteins have 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 (Asokan et al 2012, Mahon et al 2012). In that regard, one strategy involves the presentation of several toxins together, especially if a differing mode of action involving different receptors is available (Meserati et al 2005).

The *vip3Aa19* gene, described in this stacked event, is a modified version of the native *vip3Aa1* gene (Estruch et al, 1996) found in the *Bacillus thuringiens* strain AB88. It encodes a Vip3Aa19 protein that differs from the Vip3Aa1 protein encoded by the *vip3Aa1* gene by a single amino acid at position 284. The *vip3Aa1* gene encodes lysine at position 284 and the *vip3Aa19* gene encodes glutamine.

However, the applicant continually refers to the Vip3Aa20 protein through the dossier. Compared to the Vip3Aa19 protein, Vip3Aa20 also differs from Vip3Aa1 at position 284 and encodes glutamine residue instead of lysine at this position and in addition, Vip3Aa20 has an additional difference from vip3Aa1 at position 129, where an isoleucine residue has replaced a methionine residue (Raybould and Vlachos, 2011).

The applicant states that the activity of Vip3Aa is limited to Lepidopteran insects, providing as their reference the *Bacillus thuringiensis* toxin specificity database (http://www.glfc.forestry.ca/bacillus/). However, the only information to be found on that page regarding Vip3Aa proteins is an article which deals with their purification and toxicity to spruce budworm and gypsy moth (Milne et al, 2008), which cannot support the claim of Lepidopteran-specificity made by the applicant. Assuming that information has been removed from the site, the applicant will have to supply other references to substatiate this claim. Furthermore, a recent review by van Frankenhuyzen (2013) on the cross-order activity of *Bacillus thuringiensis* proteins found that activity of a number of these proteins was not restricted to particular insect orders as once thought. Altough this study did not document cross-order activity of Vip3Aa proteins specifically, 'lack of presence is not proof of absence' as the author put it, indicating that much more work still has to be done before conclusions can be drawn (van Frankenhuyzen, 2013).

In this stack, there are several 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 (Lee et al 2003). However, special concern or vigilance should be paid to GM stacks that combine events that have similar type of mode of action through their expressed transgenic proteins. Also, the Cry proteins can attach to the same receptor, changing their mode of action. In theory, the presence of two toxins can result in cross resistance and a changed effect on target and also non-target species (Schnepf et al 1998, Hua et al 2001, Estela et al 2004, Li et al 2004).

The expression of the Vip3Aa20 and PMI expressed in MIR162 is currently under evaluation by EFSA. Thus, waiting for their safety evaluation should be addressed as uncertainty remains of the proteins in question. Especially, an overall toxicity study of the GM stacked event should have been considered, despite the applicant's assertion that the 28 day toxicity study is not needed due to previous history on safe use of the of the proteins in the single events (Technical Dossier Part I, p 87).

For the VIP proteins, MIR 163 has previously been assessed expressing the VipAa20 protein. Previous evaluations of this event 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.

Recommendation:

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

Safety of Cry genes

As already mentioned **Bt11xMIR162xMIR604x1507x5307xGA21 maize** combines different classes of Bt proteins named Cry toxins. These toxins are claimed and believed to be safe, however lately the potential of non-target effects of Bt toxins concerning mode of action have been addressed (Gilliand et al 2002, Crickmore 2005, Hilbeck and Schmidt 2006, Mesange et al, 2012).

In relation to non-target and environmental effects, in two meta-analyses of published studies on non-target effects of Bt proteins in insects, (Lövei and Arpaia 2005) documented that 30% of studies on predators and 57% of studies on parasitoids display negative effects to Cry1Ab transgenic insecticidal proteins. A review by (Hilbeck and Schmidt 2006) on all Bt-plants found 50% of studies documenting negative effects on tested invertebrates.

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 had only 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 up to now (van Frankenhuyzen, 2013). Allowing for the fact that for practical reasons, not every potentially sensitive species can be tested for sensitivity to Bt toxins, it still 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 therof) of the toxin, among other factors (van Frankenhuyzen 2009).

Another quantitative review by (Marvier et al 2007) suggested a reduction in non-target biodiversity in some classes of invertebrates for GM (Bt) cotton fields vs. non-pesticide controls, yet found little reductions in biodiversity in others. More recent research on aquatic environments has sparked intense interest in the impact of Bt-crops on aquatic invertebrates *Daphnia magna* (Bøhn et al 2008), and caddisflies (Rosi-Marshall et al 2007). These publications warrant future study, given the potential load of novel target proteins (in combination with herbicides) that may end up in agricultural runoff and end up in aquatic environments. Further, (Douville et al. 2007) present evidence of the persistence of the *cry1Ab* transgene in aquatic environments: more than 21 days in surface water and 40 days in sediment. 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 relation to health impacts, a publication by (Dona and Arvanitoyannis 2009) reviews the potential health implications of GM foods for humans and animals, including incidences and effects of increased immunogenicity, amounts of anti-nutrients, possible pleiotropic and epigenetic effects, including possible reproductive and developmental toxicity. They conclude that while there is strong evidence for health concerns on many fronts, exposure duration many have not been long enough to uncover important effects. Studies should also include subjects with immunodeficiency or exposed to other stress agents.

Indications of harm to non-target organisms in the environment, and possible impacts to human and animal health prompted the Austrian Authorities to invoke a safeguard clause to ban the use of Cry1Ab-containing maize even MON810 (Umweltbundesamt, 2007). We

refer to this report as a detailed analysis of potential adverse effects from a Cry1Ab-producing GMO.

Recommendation:
- The regulator is encouraged to ask the applicant address the potential of non-target effects of Bt toxins, especially in the context of their combined use in a stacked event, as well as in combination with the trait-specific herbicides.
- 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.

Information relating to the genetic modification (p 17)

A. Identification and characterization
2. Molecular characterization

The use of Southern blots for the molecular characterization of the event is probably reflective of the fact that this stacked event is a combination of several older single-gene events, which were developed several years ago. Although the technique is not without its uses, Southern blotting is outdated, and other techniques have since developed which can provide more in-depth information about transgenic events. For example, next generation sequencing may improve the molecular characterization of events by providing data of the entire transcriptome, or allowing analysis of genetic variance between whole genomes of transgenic and conventional varieties (Varshney et al 2009). The use of more up-to-date techniques may help put to rest some of the residual uncertainties regarding the stability and placement of transgenes within the crop genome.

Recommendation:
- The regulator is encouraged to suggest that the Applicant replace Southern blotting with more modern techniques.

2.2.2 Information on the sequences actually inserted or deleted e) p. 47

GA21:
Bioinformatic analysis of the maize genomic sequence flanking the 5' region of the GA21 insert indicated that the insert disrupted the chloroplast gene encoding for a hypothetical cytochrome c biogenesis protein. It is stated that the presence of a functional cytochrome c biosynthesis gene in the maize chloroplast genome will compensate for the disrupted version seen in the genome, and then referred to the phenotypic and compositional analysis as proof of no disruption of cytochrome c activity. The cytochrome c protein is an electron carrier that operates in the mitochondria/chloroplasts of plants, and takes part in plant metabolism.

Recommendation:

- The regulator is encouraged to ask the Applicant to perform a stress test to see how the cyt c and GA21 performs under stress compared to unmodified genetically similar maize.

2.2.3 Information on the expression of the inserted/modified sequences (p.50-51)

The Applicant does not consider it necessary to supply data on the expression of the novel traits in Bt11xMIR162xMIR604x1507x5307xGA21 maize under the appropriate setting of the complete stacked event in combination with herbicide use referring to EFSA, 2011, section 3.1.2.2.

p.52

Statistical differences were observed between the tissues of the stacked event and the tissues of the single events for Cry1Ab, mCry3A and Cry1F.

Recommendation:

- The regulator is encouraged to ask the Applicant to supply data on the expression of the novel traits in this stack in combination with the herbicide since this is how it will be grown.
- The regulator is encouraged to ask the Applicant to comment the magnitude of the statistical difference, in what kind of tissues these differences are observed, and if it has a potential impact on the actual insect-resistance capability of the plant (stacked event) and potential for development of Cry-resistance in the targeted insects.

3. Comparative assessment (p 56)

3.3 Compositional analysis

No testing for herbicide residues has been done in the compositional analysis, testing for herbicide residues in plant tissue should be implemented as it has been shown that herbicides accumulate in herbicide resistant GM plants. Though level of accumulation will depend on the degree of herbicide use, accumulation levels for the recommended application should be given.

Recommendation:

- The regulator is encouraged to ask the Applicant to test for herbicide residues.

In several cases equivalence to the reference lines could not be established and composition components fell into the equivalence categories iii and iv with outcome types 5 and 6 (non-equivalence between GMO and the reference line is more likely than not), 7 (non-equivalence between GMO and the reference lines), 8 (equivalence cannot be determined due to a zero estimate of variance for genotype) or *(non-equivalence between the GMO and the reference lines, and between the comparator and the reference lines). This lack of equivalence is

considered biologically irrelevant since values still fall within the levels found for maize in the ILSI Crop Composition Database.

Recommendation:

- The regulator is encouraged to ask the Applicant to comment on whether the lack of equivalence could be due to the single genetic modifications (i.e. single events differ from conventional) or if there is an effect of stacking the traits (the stacked events are more different from the conventional than the single events are).

3.5 Effects of processing

In this section, the Applicant concludes that there are no alterations to the metabolic pathways and that there are no biologically significant changes to any of the natural constituents of the maize grain of the stacked event, and thus no need to perform toxicological tests on the processed product produced from the stacked event. Again, the applicant fails to acknowledge the context of use for the stacked event and its complimentary herbicide technologies. Since there is scientific peer-reviewed research (Bøhn et al 2014) showing the accumulation of herbicide in herbicide tolerant crops, toxicity tests are justified an should be performed.

Recommendation:

- The regulator is encouraged to ask the Applicant to acknowledge the context of use for the stacked event and its complimentary herbicide technologies. Toxicity tests are justified and should be performed.

4. Toxicological assessment

There is no toxicological assessment of the stacked event, but the applicant refers to assessments of other similar stacks and the single events used to build the stack in question here. Again, the applicant fails to acknowledge the appropriate context of use with several insecticidal and herbicide resistant traits together in one plant; the applicant also fails to consider the potential impact of complimentary herbicide technologies involving two separate types of herbicides (glyphosate and glufosinate-ammonium, which potentially could influence the toxicity of the plants through accumulation in plant tissue Particularly this should be covered in section 4.3 *Assessment of new consitutents other than proteins*.

Recommendation:

- The regulator is encouraged to ask the Applicant to consider the potential impact of complimentary herbicide technologies involving two separate types of herbicides (glyphosate and glufosinate-ammonium, which potentially could influence the toxicity of the plants through accumulation in plant tissue

Feeding trials using the whole stack to investigate for potential toxicological, allergenic or combinatorial effects

EFSA has previously found that the single events of Bt11, MIR162, MIR604, 1507 and GA21 as safe as their conventional counterparts for consumption (human and animal).
Feeding with the single events in poultry for 7 weeks have been performed, but not with the whole stack. Thus, a potential combinatorial effect of the different proteins (as well with the trait-specific herbicides) have not been investigated properly. Due to the data obtained from the single events, the Applicant do not find it necessary to perform any additional testing of the proteins expressed in the whole stack, in combination as there are "no indications of potential interactions". However, interactions with toxins expressed in stacked traits can not be excluded, and as such, potential immunological or adjuvant effects has been considered by others (Halpin 2005, de Schrijver et al, 2007). Then (2009) reviews and discusses the evidence for changes in activity and specificity of Bt proteins dependent on synergistic interactions with extrinsic features. Such changes may critically influence the bioactivity and hence the potential for unintended effects.

Recommendation:

- The regulator is encouraged to ask the Applicant to provide data for analysis of potential combinatory effects, including those of the herbicides, by the use of appropriate feeding trials.

Data for allergenicity is provided for the single events constituting the stack Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 and EFSA has found the potential for the single proteins to be allergenic for absent. The potential for an allergenic reaction to a combination of these proteins is commented to be unlikely, but has not been tested. One of the proteins, PMI (Phosphomannose isomerase) came up with a continuous stretch of 8 amino acids being equal to that of an allergen (α-parvalbumin). However, serum analysis of this single protein for evaluation of an allergenic reaction revealed that this equivalence was not biologically significant. The potential for these proteins to be parts of protein complexes and as such be potential allergens have not been elucidated. Also, the combination of the proteins in the stack should be analysed in combination using serum screening. This is however not found to be necessary.

The gastric enzyme degradation essay was performed for each of the transgenic proteins, one by one, and not of the transgenic proteins in combination. It is therefore unclear if these proteins can protect from rapid degradation when assessed in combination and as they are expressed in the transgenic plant.

Recommendation:

- The regulator is encouraged to ask the Applicant is recommended to analyze for allergenicity of the proteins in combination using SGF and also serum screening analysis.

E. Environmental risk assessment (ERA)

The application in question is for the import, processing and use of the stacked event Bt11xMIR162xMIR604x1507x5307xGA21, not cultivation. Thus the environmental risk assessment presented in the application is only concerning potential environmental risk in Europe.

However, the Norwegian Gene Technology Act states that the production and use of genetically modified organisms should take place in an ethical and socially justifiable way, and in accordance with the principle of sustainable development and without detrimental effects on the environment. With this in mind, it is our belief that the entire life cycle of the organism and the potential environmental risk it could pose to biodiversity and the environment should be evaluated. Thus we find the ERA lacking in that it does not consider risks connected to the cultivation of the Bt11xMIR162xMIR604x1507x5307xGA21 maize.

Beneath several considerations that should be taken into account are listed:

- In section 3.3.1 of the ERA the applicant continuously refer to an article by van Frankenhuyzen regarding the insecticidal activity of crystal proteins (van Frankenhuyzen K 2009), and claiming based on this article that Cry1Ab, Vip3Aa1, mCry3A , eCry3.1b and Cry1F is specific to their targeted order of insects (Lepidoptera or Coleoptera) and that no effects on other orders of organisms have been reported. However, this is a severe misinterpretation of the van Frankenhuyzen article which quite clearly states that current knowledge on protein specificity *"…is restricted by the range of toxins tested to date and the range of species used in those tests.*" Additionally the test data presented in the article is among other limitations limited to only three insect orders, the Lepidoptera, Diptera and Coleoptera, thus not including the 21 other orders of insects, or other organisms such as aquatic invertebrates. Finally, the article does not provide data on the Vip3Aa or any Vip proteins, even though it is clearly referenced in the section on MIR162 maize on page 147. Nor are there any specific mentions of mCry3A or eCry3.1b in the article, though closely similar proteins are included in the data presented. Furthermore, a more recent review by the same author (van Frankenhuyzen, 2013) on the cross-order activity of *Bacillus thuringiensis* proteins found that activity of a number of these proteins was not restricted to particular insect orders as once thought. Declaring no non-target effects on any of the millions of living organisms is not (and probably never will be) substantiated by scientific literature.
- Twice in the dossier (page 38 and page 148), the Applicant makes the statement that, regarding the eCry3.1Ab protein, '*no biological activity has been observed in tests of multiple other organisms, including lepidopteran insects, non-target insects, and avian, mammalian, and aquatic species.'* This sweeping statement was made (and repeated) without an attempt at providing a single reference to substatiate any part of it. Where is this data to be found so that it may support this claim?
- The risk of combinatorial effects of the five crystal proteins produced by the stack, on non-target organisms should be considered in the ERA.
- The potential development of cross-resistance between the transgenic insecticidal traits should be considered in the ERA.

- Considering the increasing development of resistance to the herbicide glyphosate (Heap, 2014), and the fact that the use of the herbicide glufosinate is all but banned in the EU due to health risks, neither of the co-technology options for weed control makes a convincing case for responsible and sustainable agricultural practices.
- There is a general lack of long-term studies of the effect of exposure to crystal proteins on non-target organisms. Most studies are performed over timeframes that do not imitate true exposure periods. Additionally studies on the effect of simultaneous exposure to multiple insecticidal proteins (cocktail effect) are few and far between, and there are concerns related to whether effects are additive or not, and if they are additive to what degree.

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 Norwegian Gene Technology Act, 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 detailed in the regulation on consequence assessment section 17 and its annex 4.

The NGTA, with its clauses on societal utility and sustainable development, comes into play with a view also to health, environmental and socio-economic effects in other countries, such as where GMOs are grown. The application does only concern import, food and feed use and processing of Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21. Hence, it is not intended for cultivation in Europe or Norway. This GM maize is, however, not yet approved for cultivation in any third country. Information for the risk assessment on the cultivation, management and harvesting stages as well as the post-market environmental monitoring in the producing country is required in order to assess the sustainability criteria laid down in the Act. Hence, a proper evaluation of potential impacts of relevance to sustainability can not be completed until the GM maize has been approved for cultivation in a third country, and relevant information has been provided.

The evaluation of cotechnology, that is, secondary products that are intended to be used in conjunction with the GMO, is also considered important in the risk assessment of a GMO (Dolezel et al 2009). Therefore, considerations of the co-products also warrant an evaluation of safe use. The Bt11xMIR162xMIR604x1507x5307xGA21 maize confers tolerance to herbicides containing glufosinate-ammonium and glyphosate. Glufosinate-amonium is a class of herbicides that are banned in Norway and in EU (except a limited use on apples) due to both acute and chronic effects on mammals including humans. Moreover, there is conflicting evidence with regard to whether the cultivation of herbicide tolerant GM crops has resulted in reduced use of herbicides or not. For instance, several reports show that while there was an decrease in the use of herbicides after herbicide tolerant GM crops where introduced in the US in the mid 1990s, there has been an increase in the herbicide use since around 2006, so that current herbicide use exceeds the level of herbicide usage at the time when herbicide

tolerant GM crops were introduced (Benbrook, 2012; Bonny, 2011; Food and Waterwatch, 2013). Aditionally, studies has shown increased levels of herbicide residues in herbicide tolerant GM crops (Duke et al. 2003; 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.

The Applicant has not provided information on the contribution of the Bt11xMIR162xMIR604x1507x5307xGA21 maize to the emergence of glyphosate and glufosinate-ammonium resistance in weeds, nor if there are already cases of this in the areas intented to cultivate the variety. Weed resistance to glycines in maize cultivation has been vastly documented[1]. Evaluation of the occurence of volunteer plants and suggested control strategies is also relevant. As stated by the Applicant (part 3.1, page 130): "*Survival of maize is dependent upon temperature, seed moisture, genotype, husk protection and stage of development. Maize is not a persistent weed. Maize seed can only survive under a narrow range of climatic conditions. Volunteers are killed by frost or easily controlled by current agronomic practices, including ploughing and the use of selective herbicides."* Information about which herbicides that will be used is required to evaluate potential health and environmental impacts of these.

Bt11xMIR162xMIR604x1507x5307xGA21 maize does also confer resistance to certain lepidopteran and coleopteran pests. Resistance development among target pests of Bt maize has been documented (Van den Berg et al., 2013; Van den Berg, 2013). Hence, evaluation of resistance development within the target pest population and strategies suggested to halt this development is crucial in a sustainability assessment. As emphasised by the Applicant (part 3.3. page 146): *"resistance development is only relevant for applications with scope cultivation of GM plants and not for applications restricted to import and processing of GM plants and their products"* according to EFSA ERA guidance. The applicant does therefore not include any information of relevance to this. Again, this information is however required in order to meet the requirements for sustainability assessment as laid out in the NGTA.

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 the socio-economic aspects related to the cultivation (and to a much lesser extend, the use) of GM maize. It is in any case difficult to extrapolate on hazards or risks taken from data generated under different ecological, biological, genetic and socio-economic contexts as regional growing environments, scales of farm fields, crop management practices, genetic background, interactions between cultivated crops, and surrounding biodiversity are all likely to affect the outcomes. Hence it cannot be expected that the same effects will apply between different environments and across continents. Hence, the applicant does not attempt to identify socio-economic implications, nor demostrate a a benefit to the community and a contribution to sustainable development from the use of the Bt11xMIR162xMIR604x1507x5307xGA21 maize.

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

It is also important to evaluate whether alternative options (e.g. the parental non-GM version of this Bt11xMIR162xMIR604x1507x5307xGA21 maize) may achieve the same outcomes in a safer and ethically justified way.

Ethical considerations

While it is understood that the Applicant has not applied for deliberate release of Bt11xMIR162xMIR604x1507x5307xGA21 maize in Norway, the acceptance of a product in which the intended use involves the use of a product banned in Norway, as the glyphosinate-ammonium, would violate basic ethical and social utility criteria, as laid out in the NGTA. That is, we find that it would be ethically incongruous to support a double standard of safety for Norway on one hand, and safety for countries from which Norway may import its food and feed on the other. This line of reasoning is consistent with the provisions under the NGTA to assess ethical, social utility and sustainable development criteria not only for Norway, but for countries from which Norway imports food and feed. Specifically, this issue is relevant particularly in revised regulations of 2005 Section 17 "Other consequences of the production and use of genetically modified organisms" points 2 and 3 "*ethical considerations that may arise in connection with the use of the genetically modified organism(s)*», and "*any favorable or unfavorable social consequences that may arise from the use of the genetically modified organism(s)*", respectively.

Recommendations:

- The regulator is encouraged to ask the Applicant to submit required information on the social utility of Bt11xMIR162xMIR604x1507x5307xGA21 maize and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

- The regulator is encouraged to ask the Applicant to submit required information on authorised, alternative and sustainable methods to control volunteers in Europe.

GenØk previously comments on sub-combinations and single events of **Bt11xMIR162xMIR604x1507x5307xGA21 maize** can be found in:

- EFSA/GMO/DE/2010/86 for Bt11xMIR162x1507xGA21 (our previous comments from July 2012: **H_86**)
- EFSA/GMO/DE/2011/95 for 5307 (our previous comments from August 2011: **H_95**)
- EFSA/GMO/UK/2010/83 for MIR604 (our previous comments from March 2011: **H_83**)

*Application: EFSA/GMO/DE/2010/86 for Bt11xMIR162x1507xGA21 (our previous comments from July 2012: **H_86)***

Molecular characterization:

Assessment of the newly expressed protein
Analysis of the proteins expressed in the stack Bt11xMIR162x1507xGA21 was done by comparing expression levels to the corresponding single events. The proteins were not isolated from herbicide treated plants. This should have been included as a control.

The applicant stated on page 56, last paragraph "the results obtained demonstrate that the levels of expression of the transgenic proteins in Bt11 x MIR162 x 1507 x GA21 are comparable to the levels of expression of those proteins in the four single maize events." Also on page 57 that "Mean Cry1Ab protein concentrations were comparable in the tissues of Bt11 maize and Bt11 x MIR162 x 1507 x GA21 maize". However, in Table D.3(a)-1 on Page 59, the mean difference of Cry1Ab concentration between the single and combined events are not comparable in Leaves (V10-10 Leaves) and in Leaves (R1 Silking). Similarly the mean concentration of Vip3Aa20 between the single and stacked events in Leaves (V10-10 Leaves) are anything but comparable; the same is true for mEPSPS mean concentration comparison in Leaves (R1 Silking) between the single and stacked events.

Similarly, the mean PAT protein concentrations between the single stacked events shown in Table D. 3(a)-1 (continued); page 61 are not additive between the events. What is the aim of comparing the protein expressions between the events if there is no additivity between the two single events and the stacked events? For example, in (V10-10 Leaves) the PAT protein concentration in the single event Bt11 (0.31µg/g) and the single event 1507 (4.72µg/g) comparably adds up to the protein concentration of the stacked event (4.95µg/g). However, in the (R1 Silking) PAT protein concentration in the single event 1507 (6.26µg/g) is higher than the combined concentration of the protein in stacked event (4.72µg/g) and single event Bt11 (0.58µg/g). This shows clearly that the PAT protein expression in the stacked event is not comparable to the individual single events. More information and better clarifications are required to rule out unforeseen aberrant regulations of protein expression.

On page 58 paragraph 2 the applicant stated "Although some statistically significant differences were seen, **these differences were small or not consistent** across the growing

season". This statement does not provide valid explanation(s) for the discrepancies itemized above. In this case, a **difference between data cannot be both characterized statistically significant and small**.

Comparability of quantities of expressed proteins does not show that the compared proteins are similar. Thus, the applicant needs to provide data showing that the individual proteins from stacked events have not differed in amino acid sequences from equivalent proteins produced in single events. This would rule out that isoforms of the relevant proteins are not expressed under the stacked event.

Recommendation:

- The applicant should give explanations/clarifications for observed statistically significant differences? What is (are) biological relevance of these differences?
- The Applicant should provide standard deviation alongside the means
- The Applicant should provide data showing that the individual proteins from stacked events have not differed in amino acid sequences from equivalent proteins produced in single events

Allergenicity assessment

In case the GM crop will be used for animal (feed) or human nutrition (food), the risk assessment should contain additional information on toxicological, allergenic and nutritional food/feed aspects (EC, 2003a). However, the applicant claims that a 28 day toxicity study is not needed due to previous history of maize and the analysis made of the single proteins in the stack indicating no homology to know allergens, lack of acute toxicity, rapid digestion and the consideration "non-toxic and unlikely to present health risk to humans or animals".

One sequence alignment of eight continuous stretches of identical amino acids between PMI and a known allergen was identified. Serum screening analysis resulted in no detectable cross reactivity. However, the combined effect of potential allergens in the stack has not been even theoretically considered as a possibility or "has not been investigated yet". This seems not to be considered as a potential risk by the applicant.

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, Rojas-Hernandez et al, 2004). 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 combined effect of potential allergens in the stack should be investigated.

Toxicological assessment
Due to the history of safe use, the same points are used as for the allergenicity assessment. However, one of the single events, MIR 162 has not been fully evaluated by EFSA and specific concern or attention should be set on the toxicity assessment from them.
For GM stacked events there has not been enough evaluation of the potential for change in expression level of the different proteins as compared to the single events. And according to Kuiper et al (2001), the information on the expression level of the transgenic proteins in the stacked event is relevant when considering the need for whole GM food/feed toxicology studies of the GM stacked event. However, it should be realized that such whole food testing experiments have their limitations, due to limited dose range and complexity of the product.
Potential interaction between the newly expressed proteins is not investigated. The possibility of interactions and resulting toxicity/allergenicity aspects are not mentioned. No tests are however available to predict such interactions at the cellular level. But additional toxicity studies could be performed.

Recommendation:
- Since one of the single events, MIR162 has not been fully evaluated by EFSA, specific concern or attention should be given to their toxicity assessment

*Application: EFSA/GMO/DE/2011/95 for 5307 (our previous comments from August 2011: **H_95**)*

1.1 Assumption of safety of the chimeric Cry3A-Cry1AB transgenic protein in 5307 based on unassociated prior evaluations of Cry3A and Cry1Ab proteins

The Applicant states that:
"The eCry3.1Ab protein is a chimeric protein based in a modified Cry3A protein (mCry3A), derived from the Cry3A protein from B.thuringensis subst. tenebrionis, and the Cry1Ab protein from B. thuringiensis sunst kurstaki HD-1. The safety of the mCry3A and Cry1Ab proteins has been assessed by EFSA as part of the evaluation conducted for the MIR604 maize import application (EFSA, 2009a) and Bt11 maize renewal (EFSA, 2009b), respectively. (p.9)"
However, the conclusion of safety of a chimeric version of a separate protein is not scientifically valid. A number of structural or functional features of the protein, particularly immunogenic or toxicological properties may have changed in the chimera, which should be investigated with a new protein characterization of the bioactivity of this protein, and should be reflected in toxicological and functional tests.

Recommendation: We recommend that the Norwegian Environment Agency request information from the Applicant related to potential functional and structural changes that may have occurred in the production of the chimeric Cry3A-Cry1AB transgenic protein in 5307.

2. Missing, incomplete or inadequate information to support the Applicants calims
2.1 Detection and absence of backbone vector DNA/unintended transgenes in 5307

In Appendix I (p.56) the applicant describes the use of a 5312 bp "backbone probe" for detecting possible Integration of backbone sequneces into 5307.
Backbone transfer are common when Introducing recombinant DNA using the Ti plasmid system found in Agrobacterium. Historical sdata underestimates the number og back bone transfer becausee: "usually, transfer of the only non-T-DNA sequences to the plant would remain undetected becausee:1) there Is no selection for the transfer og such sequences" (Kononov et al 1997). The amount of DNA that can transfer van be many times the length of the T-DNA region: " extremel long regions of DNA (greater than 200 kbp) can transfer to an Integrate into the genome of plants" (Kononov et al 1997). Short back bone sequences can transfer and be difficult to detect. "in many instances, vector backbone regions of a binary vector are smaller than what is conventionally termed the T-DNA region (Kononov et al 1997). The Applicant used Southern blotting to raise confidence In the conclusion that there were no insertions of unintended maerial. Unfortunately, in this case only one probe (5312 bp) corresponding to the entire backbone sequence was used. Such large probes are prone to give false negative results becausee small Inserts would not retain the probe during high stringency washing of the blot (65°C, 0.5-2 x SSC). The Applicant has not justified this stringency and has not validated It for surveying this genome (see above). The Applicant should have used a comprehensive set of smaller probes.

Taking together the above problems In methology and reporting, there is insufficient evidence to calim "5307 maize Is free of backbone sequence from the transformation plasmid pSYN12274." (p. 56 terchnical dossier)

Recommendation: The Norwegian Environment Agency should request additional data using a comprehensive set of smaller probes to establish the presence or absence of backbone vector DNA sequences at a limit of detection of ≤ one target/tetraploid genome.

2.2 Protein characterization

First, the antigen used to raise anti-cry antibodies, and the antibodies themselves utilized in the immunoreactivity assays lack description. Based on our reading, it is not clear what the origin of the protein was that was used to raise the antibodies in the first place, or how the antibodies were purified from serum (e.g. which antigens were used to purify by immunoaffinity chromatography?). Post-translational modifications vary by species, tissue and time of development and epitopes can be masked by post-translational modifications (Kuester et al. 2001). Therefore, raising antibodies against the E. coli produced form will obviously bias all subsequent equivalence testing against the detection of potential novel inplanta produced isoforms. It is impossible to say, using the evidence provided, that the polyclonal antibodies would in fact detect all isoforms of the recombinant proteins that might be produced in-planta, were they present in the sample. A precautionary approach should conclude that the Applicant has profiled only a subset of epitopes on the unglycosylated isoform of the recombinant protein.

Recommendation: The Applicant should provide evidence that the antibodies used in the protein characterization would detect all novel in-planta produced isoforms.

Application: EFSA/GMO/UK/2010/83 for MIR604 (our previous comments from March 2011: ***H_83****)*

1.1 Approval for MIR604 sought when intended event for production and cultivation is BTxMIR604xGA21

As previously stated, the Applicant is seeking approval for MIR604 on the assumption that its successful authorization will lead to the acceptance of the same trait, along with others in combination and also under regulatory consideration independently in the EU, towards the approval of a new "stacked event", in separate submission. EFSA/GMO/UK/2008/56. The Applicant indicates its intention is note for the commercial use of MIR604, but rather Bt11xMIR604xGA21 maize stack. We do not agree with the basic premise that approval of MIR604 is preliminary to the full consideration of Bt11xMIR604xGA21, as both will require a separate event specific assessment in any case. That is, whenever a new event is applied for release, even if it is the product of other events which are also under application for approval, genetic background ecological context etc., may influence or limit the value of indirect comparative assessments.

Therefore, if the Applicant has no intent to market MIR604, the assessment here is likely not to produce any value above what would be required for BtxMIR604xGA21. Hence the economy of this of this exercise, in terms of time and financial resources, in our mind, should be seriously questioned. However, we will base our information solely on the event in question, MIR604 rather than the intended event for environmental release BtxMIR604xGA21.

Recommendation: The Norwegian Environment Agency should question the value and use of resources in evaluation an application for approval where the Applicant states it does not intend to market this event, but rather an event with the target gene as part of a "stacked" event, given the necessity of a full risk appraisal of the intended "stacked" event.

2. Missing, incomplete or inadequate information to support scientifically sound claims of safety

As the event MIR604 is currently being evaluated for food, feed and processing in the EU under Application EFSA-GMO-UK-2005-11, and given that the Applicant relies heavily on the information submitted in this application, we wish to direct attention to the critiques submitted by Member states concerning the informational deficiencies and critiques in EFSA-GMO-UK-2005-11, and include input from Norway.

In summary, a number of member countries found reason to comment on deficiencies in the application, specifically related to the molecular characterization, use of "surrogate" proteins in the experimental studies, the design of the 90 day rat feeding studies, the interpretive inference from the comparative tests, the potential allergenic effects, among others. Based on member state input, we also find reason to question the veracity of the information submitted under Application EFSA-GMO-UK-2005-11, and encourage the competent authorities in Norway to follow up on their previous queries contained therein.

Specifically, based on our reading og the dossier, we could find no documents oa a more elaborated post-release monitoring plan that was provided in Application EFSA-GMO-UK-2005-11, where cultivation was not applied for. Despite the Applicant claim that "the presence of MIR604 maize in food and feed will not result in any nutritional changes, therefor post-marketing monitoring is not considered necessary" (p. 66)

We find that the application for cultivation should follow with a more detailed monitoring plan that complies with Annex VII of Directive 2001/18/EC.

Recommendation: The Norwegian Environment agency should follow up with the outstanding issues raised by member countries in the evaluation of EFSA-GMO-UK-2005-11. Specifically, the Applicant should submit a more detailed plan for post-release monitoring compliant with Annex VII of directive 2001/18/EC.

Conclusion

Available information for risk assessment evaluation

This evaluation is based on the Applicant's own submitted information, along with our own expertise in related fields. The relevant scientific literature is very limited in some cases, yet we have tried to extract information from the peer-reviewed literature that may inform the scientific validity of the information under consideration. In situations where lack of knowledge, complexity and uncertainty are high, particularly in relation to unknown adverse effects that may arise as a result of approval for release of a living modified organism into the environment or food supply, the available information may not be sufficient to warrant approval. Further information may address some of these issues, however an accurate description of uncertainties provided by the applicant would provide a more useful basis for assessing the level of risk that may come with regulatory approval of the GMO, taken on a case-by-case basis.

In all cases, product-related safety testing should have an independent and unbiased character. This goes both for the production of data for risk assessment, and for the evaluation of the data.

The lack of compelling or complete scientific information to support the claims of the Applicant documented here highlights the need for independent evaluation of the dossier as performed here, including the raw data produced by the Applicant. We therefore support better transparency and independent review of information to ensure high standards within the regulatory process. This would include any information provided by the Applicant used to justify confidentiality claims on any scientific data. We encourage the authorities to insist on this level of transparency and accessibility to all scientific data (including raw data) to ensure the scientific validity of the information presented.

Overall recommendation

Above we highlight a number of issues in relation to the questionable safe use **Bt11xMIR162xMIR604x1507x5307xGA21 maize** that do not justify a conclusion of safe use, social utility and contribution to sustainable development. Critically, the Applicant's environmental monitoring plan lacks sufficient details and descriptions to support the required monitoring activities, and 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. Taken together, these deficiencies fail to address the necessary safety regulations under Norwegian Law, and thus the application is incomplete and should not be approved. 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 **Bt11xMIR162xMIR604x1507x5307xGA21 maize** we conclude that based on the available data, the Applicant has not substantiated claims of safety satisfactorily to warrant approval in Norway at this time.

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