GenØk - Centre for Biosafety

Vår ref:2012/h92
Deres ref: **2012/1569 ART-BI-DHT**

Direktoratet for naturforvaltning
Tungasletta 2
7485 Trondheim
Dato: 01.04.2012

Vedlagt er inspill fra GenØk – Senter for Biosikkerhet om høringer EFSA/GMO/NL/2011/92 for 1507x59122xMON810xNK603 fra Pioneer Hi-Bred International, Inc.

Hvis du har noe spørsmål, vennligst ta kontakt.

Med vennlig hilsen,

Dr. Lise Nordgård
Forsker
GenØk – Senter for Biosikkerhet

Bidragsytere:

Dr. Idun Merete Grønsberg
Forsker
GenØk – Senter for Biosikkerhet

Jan Husby
Senion Rådgiver
GenØk – Senter for Biosikkerhet

GenØk - Centre for Biosafety

Vår ref:2012/h92
Deres ref: **2012/1569 ART-BI-DHT**

Assessment of the technical dossier submitted under EFSA/GMO/NL/2011/92maize for approval of transgenic crop, 1507x59122xMON810xNK603 from Pioneer Hi-Bred International, Inc.

Submitted to

Direktoratet for Naturforvaltning

By
Lise Nordgård, Idun Merete Grønsberg, and Jan Husby

Centre for Biosafety – GenØk
April 2012

KONKLUSJON PÅ NORSK

Vi trekker frem flere begrepsmessige, empiriske og informatoriske mangler i dossieret som ikke gir grunnlag for en konklusjon om sikker bruk, samfunnsnytten og bidrag til bærekraftighet av 1507x59122xMON810xNK603. Søker har ikke inkludert noe av den informasjonen omkring samfunnsnytten og bærekraftighet til 1507x59122xMON810xNK603 som kreves i den norske genteknologiloven (Appendix 4) for godkjenning i Norge.

Hovedkonklusjon og anbefalinger

Genøk –Senter for Biosikkerhet viser til brev fra Direktoratet for naturforvaltning (DN) angående høring relatert til maisplanten 1507 x 59122 x MON 810 x NK603 for bruksområdene import, prosessering, mat og fôr.

Maisplanten 1507 x 59122 x MON 810 x NK603 er en stablet hybrid (”multistack”) med ulike pesticid-kodende gener (Bt-toksiner) innebygd. I tillegg er den tolerant for sprøytemidler glyfosat og glufosinat-ammonium. Ingen av foreldrelinjene er godkjent for omsetning i Norge

Stablede hybridplanter har generelt en mer kompleks genetisk sammensetning og derfor større potensial for opp- og nedregulering av plantens egne gener. Det kan ikke utelukkes at gruppen av de uttrykte toksinene i planten kan gi spesifikke immunogene effekter eller adjuvanseffekter (fremming av immunreaksjoner mot andre stoffer) hos pattedyr og mennesker. Derfor mener GenØk at de burde de gjennomgå grundig testing før eventuell markedsadgang.

Informasjonen som er tilgjengelig fra søker er ikke tilstrekkelig for uavhengig evaluering av søknaden. Det foreligger ingen resultater fra analyser eller detaljerte forsøksoppsett til oppklaring av DNA sekvens, lokalisering av transgenet i maisgenomet, protein uttrykk, toksikologiske/immunologiske effekter eller foringsforsøk i relevante dyremodeller.

Søker gir ikke opplysninger som adresserer vurderingskriteriene bærekraft, samfunnssnytte og etiske aspekter som forutsettes anvendt i den norske genteknologiloven. I denne sammenheng er det viktig å få dokumentert om den omsøkte planten fører til mindre bruk av sprøytemidler, samt erfaringer med hensyn på effekter på miljø, helse og samfunnsaspekter hos bønder som dyrker den. Denne type dokumentasjon er ikke vedlagt søknad om omsetting av maisplanten 1507 x 59122 x MON 810 x NK603.

Basert på manglende uavhengige studier og data tilgjengelig ønsker vi å påpeke at det er kunnskapshull relatert til risiko for helse og miljø ved maisplanten 1507 x 59122 x MON 810 x NK603. Disse kunnskapshullene er spesielt relatert til usikkerhet ved effekter som kan oppstå på grunn av kombinasjonen eller synergistiske effekter av de innsatte genene og viser til at en bør bruke føre-var prinsippet og ikke godkjenne bruk i Norge.

Søker har heller ikke fremskaffet noe av den informasjonen som er nødvendig for å kunne

vurdere samfunnsnytte og bærekraftighet, noe som er påkrevd i den norske genteknologiloven for godkjenning i Norge. Disse manglene gjør at vi mener at denne søknaden er ufullstendig i sin nåværende form. Vi anbefaler derfor å avslå søknaden.

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av GM maisen i de bruksområder det søkes om. Konklusjonen er basert på i) manglende dokumentasjon av helse og miljøeffekter ved bruk av sprøytemidlene glufosinat og glufosinat ammonium, iv) bruken av føre-var prinsippet ved kunnskapshull og vitenskapelig usikkerhet.

SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL/2011/92

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 1507 x 59122 x MON 810 x NK603, setting out the risk of adverse effects on the environment and health, including other consequences of proposed release under the pertinent Norwegian regulations.

This submission is structured to address specific provisions for an impact assessment required under the Norwegian Gene Technology Act of April 1993, focusing on the requirements in Appendix 2 - Principles for environmental risk assessment pursuant to sections 13-16 of the regulations, and Appendix 4 - Evaluation of ethical considerations, sustainability and benefit to society, cf section 17 of the "Regulations relating to impact assessment pursuant to the Gene Technology Act" of December 2005, pursuant to section 11 cf section 8. The information presented here may be applicable to more than one provision in different appendices.

We have targeted our critique to address the information needs under the relevant provisions that relate to our particular area of competence in biotechnology assessment as comprehensively as possible. Lack of commentary on our part towards any information under consideration should not be interpreted as specific endorsement of that information.

This submission was built in large part using the **Biosafety Assessment Tool** (https://bat.genok.org/bat/) produced by the University of Canterbury and GenØk – Centre for Biosafety. This is a free-to-the-public resource for hazard identification and risk assessment of genetically modified organisms.

All page numbers following quoted text that is not directly referenced refers to the technical dossier "APPLICATION FOR AUTHORISATION OF GENETICALLY MODIFIED PLANTS AND DERIVED FOOD AND FEED IN ACCORDANCE WITH REGULATION (EC) No 1829/2003", submitted by the Applicant.

Key findings

After a analysis of many of the portions of the dossier on 1507x59122xMON810xNK603 submitted by the Applicant, we outline a number of inadequacies in the information submitted in the dossier that do not justify the Applicant's conclusion of safety. Our input focuses on a critique of the Applicant's dossier and covers two broad issues:

1. Improper assumptions, reasoning, or interpretations of data that do not support a the conclusions given, or other insufficient or missing information and/or data by the Applicant related to the dossier

2. Missing or insufficient information in relation to requirements under the Norwegian Gene Technology Act

Within each specific point we make a recommendation on the appropriate action to address the deficiencies where possible. In the concluding section of our assessment is an overall recommendation on the decision for approval.

Lastly, Codex Alimentarius guidelines allow Norway to ask for specific data of the type we identify and recommend obtaining. Norway therefore may request such information without concern of a challenge from the World Trade Organisation.

Recommendations

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

The Direktoratet for naturforvaltning is encouraged to request the following:

1. The Applicant should demonstrate the lack of interactive effects between transgenic proteins through proper scientific testing and evidence gathering, rather than justify the lack of testing based on assumptions-based reasoning of no effects.
2. The regulator is encouraged to consider the safety of co-products (e.g. herbicides) intended to be used with the GM event in the evaluation of safety.
3. A full molecular characterization, including expression levels of each of the transgene products should be performed for this event.
4. The Applicant should provide evidence of a lack of toxicological effects from interactions of the newly expressed transgenic proteins in the event under consideration in relation to their singular events.
5. The Applicant should provide scientific evidence of compositional equivalence of the newly expressed transgenic proteins in the event under consideration in relation to their singular events.
6. The Applicant should provide scientific evidence of a lack of immunogenic effects from interactions of the newly expressed transgenic proteins in the event under consideration in relation to their singular events.
7. The Applicant should follow EFSA guidelines regarding statistical significance and confidence intervals, including statistical power in the reporting of results.
8. The Applicant should submit required information on the social utility of 1507 x 59122 x MON 810 x NK603 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

Overall recommendation

Based on our detailed assessment, we find that the informational, empirical and deductive deficiencies identified in the dossier do not support claims of safe use, social utility and contribution to sustainable development of 1507 x 59122 x MON 810 x NK603. **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.

Assumption-based reasoning should not be substituted for scientific testing to bring greater certainty to the assessment and confidence in the results. Therefore, in our assessment of 1507 x 59122 x MON 810 x NK603, we conclude that based on the available data, including the safety data supplied by the Applicant, the Applicant has not substantiated claims of 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/NL/2011/92

About the event

The genetically modified maize line 1507 x 59122 x MON 810 x NK603 has been produced by conventional breeding between 1507 maize, 59122 maize, Mon810 maize, and NK603 maize. If more than one gene from another organism has been transferred, the created GMO has stacked genes (or stacked traits), and is called a **gene stacked event** like in this case.

The combined trait product expresses the following proteins: *Cry1F, Cry34Ab1, Cry35Ab1, Cry1Ab, PAT* and *CP4 EPSPS.*

These proteins give resistance against certain Lepidoptera pests (*Cry1A* and *Cry1Ab*), protection against corn rootworm larvae (*CRY34Ab1* and *CRY35Ab1*) and tolerance to the glufosinate-ammonium and glyphosate herbicides (*PAT* and *CP4EPSPS*).

Assessment findings

1.1 Assumptions-based reasoning on 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 plants own genes. Interactions with stacked traits cannot be excluded that the group of expressed toxins in the plant can give specific immunological effects or adjuvant effects in mammals (Halpin 2005, Schrijver et al, 2006). 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.

This is why combinatorial, synergistic effects must be carefully considered in the development and risk assessments of stacked events and robust data are necessary to identify whether the combined presence of transgenes influences expression levels, e.g. by silencing 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. That 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 (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.

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

1.2 Herbicides as co-products

The evaluation of co-products, that is, secondary products that are specifically designed and intended to be used in conjunction with the GMO, is 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, particularly when there is precedence in policy concerning its used independently.

The genetically modified maize line 1507 x 59122 x MON 810 x NK603 has been modified to tolerate higher doses of the herbicide glyphosate and glufosinate-ammonium.

Glyphosate tolerance (NK603)
The NK603 contains the *CP4EPSPS* gene from *Agrobacterium sp. line CP4* that confers tolerance to herbicides containing glyphosate. In recent years glyphosate has received more risk-related attention due to 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).

Glufosinate-ammonium tolerance (1507 maize, 59122 maize)
The events 1507 and 59122 contain the *pat* gene from *Streptomyces viridochromogenes* that confers tolerance to herbicides containing glufosinate-ammonium, a class of herbicides that are banned in Norway and in EU (except a limited use on apples) due to both acute and chronic effects on mammals including humans. Glufosinat ammonium is harmful by inhalation, swallowing and by skin contact. Serious health risks may result from exposure over time. Effects on humans and mammals include potential damage to brain, reproduction including effects on embryos, and negative effects on biodiversity in environments where glufosinate ammonium is used (Hung 2007; Matsumura et al. 2001; Schulte-Hermann et al. 2006; Watanabe and Sano 1998). According to EFSA, the use of glufosinate ammonium will lead to exposures that exceed acceptable exposure levels during application.

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

1.1 Molecular characterization

Maize 1507 x 59122 x MON810 x NK630 is a GM plant containing stacked transformation event of the maize events 1507, 59122, MON810 and NK630.There is no scientific literature available on the full genetic construct, the genetic stability, transgene expression products or immune-toxicological effects, or cross resistance effects unique to the stacked event in question in order to make an appropriate scientific evaluation.

The data submitted by the applicant to conclude molecular equivalence of GM maize 1507 x 59122 x MON810 x NK630 with the parental GM lines consist of *Southern Blot Analysis* of the introduced traits to confirm the maintenance of the transgenic insert of the GM parental lines, comprising their copy number and the structure. Critical here is the size of the probes where 10 of 12 probes are small, > 500bp, which means that point mutations, small deletions and rearrangements that might occur during breeding will possible not be detected (Fagard&Vauvheret, 2000, de Schrijver et al 2006).

The limited scope of the analysis as presented does not allow a comprehensive and meaningful examination of the inserts present in GM Maize 1507 x 59122 x MON810 x NK630.

Recommendation: A full molecular characterization, including expression levels of each of the transgene products should be performed for this event.

1.2 Health effects

Regarding potential health effects, the applicant claims safe use since maize in general is extensively cultivated in the EU and thus has a long history of safe use. The applicant also claims that the event is not considered to have toxic effects to humans, animals and other organisms. However, the data provided in the dossier do not give enough evidence that the use of 1507 x 59122 x MON 810 x NK603 is uniquely safe from a toxicological and allergenic point of view. Once more, no scientific studies about the stacked event in question are available in order to make an appropriate scientific evaluation (only data from singular events used in the stack are provided).

1.2.1 Safety assessment of the newly expressed proteins

The stacked event expresses Cry1F, Cry34Ab1, Cry35Ab1, Cry1Ab, PAT and C4EPSPS. Safety assessment of single proteins has been performed in previous single submissions (previous use, mode of action, specificity, biological activity, relation to other proteins with a history of safe use, absence of toxicity to mammals, absence of adverse effects on fast growing species, lack of homology to known toxins, lack of resistance to proteolysis, and degradation upon heating). According to the analysis performed by the applicant, there is no presence of similarity to proteins or any potential metabolites that could be toxic. As their evaluations has been performed with the bacterial version of the proteins only, the aim must be to use the plant derived version of the proteins in the combinations as they are in the

stacked event. This means that the protein that actually is expressed in the gene modified species, and derived from it, should be used due to the potential differences that can arise because of post translational differences between species, tissues and stages of development (Gomord et al 2005, Küster et al 2001). The fact that the maize plant has 6 "distinct" transgenes expressed at the same time in a totally new context (bacterially derived proteins expressed in plant) should be taken into consideration as this is relatively new. The translational and post translational machinery and the potential creation of new protein complexes, potential new proteins (recombinations) and potential allergic responses to that is not considered. The applicant has also not considered the potential adjuvancy of the Cry proteins expressed here. This issue was raised to the EFSA GMO Panel/Unit (EFSA/GMO/472), but not considered in this application that has 4 different Cry proteins expressed. In a report by Guimaraes et al (2008) they demonstrate the possible adjuvant properties of Cry1Ab and elicitation of the allergic reaction in a mouse model. This issue should be considered further.

According to the applicant EFSA has no safety concerns regarding any interactions between the two enzymes C4EPSPS and PAT as they have been evaluated in previous stacks that include the transgenic proteins in questions (EFSA 2008, EFSA 2009d).
The interaction between the different Cry proteins (Cry1Ab, Cry34Ab1/Cry35Ab1) has yet not been specifically assessed by EFSA. Neither has the interaction between Cry1F and Cry1Ab. The applicant states that there is no evidence for interactions between the proteins, and that *Bacillus Thuringiensis* strains normally display a variety of cry genes in various combinations (Martinez et al 2005) and that there are no health effects of these.
They have however not tested the whole plant (the stacked event) and its related proteins in combination.

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

1.2.2 Testing of whole GM food/feed
No testing of the whole GM food/feed (maize stack 1507x59122xMon810xNK603) was performed by the applicant. This was justified by the fact that the stack is found compositionally equivalent to its non-GM comparator and because the single, parental maize events indicated nothing hazardous upon a 90 day feeding study in rats (Appenzeller et al 2009, He et al 2008). The applicant should have tested the whole food/feed as it is applied for use in food/feed. A 42-day feeding study in poultry also performed but not on the stack in question, but other combinations of the parental events.
The proteins expressed in the stacked event has not been analysed by an acute toxicity test (in combination). Toxicity related effects should be looked for and the recommendation to EFSA is that a feeding study that looks for toxicity and cancer related effects should be for 1 year or more (OECD TG 451).

Recommendation: The Applicant should provide scientific evidence of compositional equivalence of the newly expressed transgenic proteins in the event under consideration in relation to their singular events.

1.2.3 Assessment of allergenicity of the newly expressed protein.
Allergenicity studies have not been performed for the stacked event in question, but on its parental maize lines, using the "weight of evidence" approach (Codex 2003, EFSA 2010). None of the newly expressed proteins have been found to have any sequence similarity to known allergens, or to have glycosylation sites indicating potential allergen. Also, the proteins are degraded fast in simulated gastric fluids, and are not heat labile (Table 12, p 145, Dossier). Of comment, is that sensitization/boosting of the allergenic response through the respiratory tract might be of higher importance in allergenic sensitization studies (commented in previous hearing, H98). The combination of all six transgenic proteins should be assessed to look at combinatorial effects. Also, the proteins analyzed are of bacterial origin and not derived from the plant itself.

None of the proteins have shown sequence similarity to known allergens when their amino acid sequences have been compared to a database of known and putative allergens. However, Cry1F was found similar to an allergen called p7 when using a frame of 6 contiguous amino acids as match criteria. However, Codex 2003 and EFSA 2010 have concluded that the frame has to be of more than 6 or 7 amino acids matches to avoid false positives.

Maize is not considered as allergenic. However, recently some maize derived proteins have been identified as allergenic; a lipid transfer protein (Pastorello et al 2003), vicilin, globulin-2 and some other proteins (Fasoli et al 2009). Maize allergies have also mainly been towards pollen. The applicant has not performed any studies of pollen from the stacked event related to allergenicity. And since the application is not for cultivation, the applicant considers this irrelevant. The applicant also used the fact that the single proteins from the parental lines have no allergenic characteristics as a conclusion that the inserted proteins are unlikely to change the allergenic potential of the stacked maize event. However, they have not tested all 6 proteins in combination through the use of the stacked event itself, only based their assumptions on the results from the single parental lines separately.

In this part of the dossier they have not considered any potential rearrangements of the genetic inserts that can cause new combinations of proteins that can have allergenic potential. There is also no mentioning or suggestions of animal studies that can give an indication of a potential immunological response to the 6 different proteins in combination. The only feeding study performed with the **whole stack** is a 42-day feeding study in poultry, but this study focused on nutrition only.

Recommendation: The Applicant should provide scientific evidence of a lack of immunogenic effects from interactions of the newly expressed transgenic proteins in the event under consideration in relation to their singular events.

1.2.4 Feeding studies
In the toxicological assessment the applicant are referring to the feeding studies with rodents with the single event maize lines and claims no adverse effects. Again, drawing conclusion on the background of experiments performed on the single events is not optimal in order to make an appropriate scientific evaluation of this stacked event.

Only one feeding study with the maize 1507 x 59122 x MON810 x NK630 has been performed. This was a 42 d poultry feeding study performed with diets containing grain from the combined trait product 1507 x 59122 x MON 810 x NK603. No differences were observed in weight gain, mortality, organ yield and carcass yield between broilers consuming diets produced with 1507 x 59122 x MON 810 x NK603 maize grain treated or untreated with the intended herbicides and those consuming diets produced with near-isoline control maize grain.

There is no data presented about the state of the different internal organs or potential microscopic and molecular in different organs or tissues or any experimental testing of the whole plant, e.g. serological testing for IgE reactivity with a representative number of sera from allergic patients which would be included in order to assess the toxicological assessment of the stacked event (Spök et al 2004).

The use of broiler chickens in general is controversial since the animal's present high mortality and diverse and severe pathological conditions as it is which means that the applicant should complement feeding studies with representative animals which are normally fed with maize, e.g. pigs.

1.3 Compositional analysis

According to the dossier, grain samples were collected from conventional or intended herbicide treated stacked maize and non-GM comparator. Then 78 different analytes were analysed (proximates, fibres, fatty acids, amino acids, minerals, vitamins, secondary nutrients and anti-metabolites). No differences to the non-GM comparator were observed except from "small differences" observed for some of the fatty acids (oleic and linoleic acid). This was however commented to be "within the tolerance interval". Also, the measured potassium level was increased in maize grain of the stacked event (treated or untreated with herbicide). These values are also commented to be "within the tolerance interval". The analyzed maize grain also contained very low levels of vitamin B2 and β-tocopherol, thus statistical analysis of these was not performable because of that. No differences were found in analyte concentration in forage of the stacked event compared to forage of control maize. No safety concerns were therefore raised due to this and after comparisons to similar data for grain and forage from the parental single event maize lines. The applicant concludes that the combination of the four single events into the 1507x59122xMon810xNK603 stacked event has no effect on the composition of grain and forage derived from it.

The use of statistically significance in deriving conclusions of equivalence or safety should be a starting point, but not an ending point in the evaluation.

Statistical analysis is central to the interpretation of scientific information and the conclusions drawn from them. The use of hypothesis testing in risk assessments commonly report on the level of statistical significance, that is, the likelihood that the result (observance of similarity of difference in the parameter measured) would be achieved by chance alone at a defined level in the analysis of the data (usually 95%). Often, an observation that reaches statistical significance is implicitly interpreted as a meaningful result, when this observation tells one

nothing about the statistical robustness of the finding. This requires a more thorough examination of the study design.

As a result, the robustness of relying on merely the reporting of statistical significance has been questioned by statisticians. Instead, the use of confidence intervals (Garder & Altman, 1986; Lee & Lovell, 2009) provide greater levels of information about the robustness of the data under examination, along with an examination of statistical power, effect sizes (Luus et al. 1989). The European Food Safety Authority (2011) outlined a number of recommendations related to the use of statistical significance.

EFSA finds that the reporting of statistical significance is not the same as a statistical analysis of the results. The latter involve one or more of the following: a description of the data, types of statistical tests used, data management and means of interpretation of quantitative data with the aim to make inferences on the underlying relationships in the data. Further, reporting of statistical significance is not alone the reporting of the output (p-value), but require the description of the study design, the data, assumptions made, and the statistical model used to test the formulated hypothesis. Whenever possible, assumptions should be tested to study the robustness of the statistical result. Further, the use of statistical significance alone cannot summarize all of the critical information in the data. Therefore, reporting of confidence intervals (see above) can give considerably more information about the effect size, its uncertainty to be able to draw accurate conclusions on the robustness of the data presented. Non-significant findings should not be used to draw conclusions of no-effect.

“Identifying statistical significance should not be the primary objective of ta statistical analysis…less emphasis should be placed upon the reporting of statistical significance and more on statistical point estimation and associated interval estimations (e.g. Confidence Intervals) as more information can be presented using the latter. In addition [EFSA] recommends that a complete description of the methods used, the programming code and the raw data are made available to the assessors so that alternative analyses could be conducted to test the robustness of the conclusions drawn. Results of statistical testing should not be dichotomised into significant and not significant. If however the results have been described as “not significant”, the study design should be explored to see whether it had sufficient statistical power to detect biologically relevant effects” (EFSA, 2011).

Recommendation: The Applicant should follow EFSA guidelines regarding statistical significance and confidence intervals, including statistical power in the reporting of results.

2. Missing or insufficient information in relation to requirements under the Norwegian Gene Technology Act

2.1. 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. 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 Applicant has not provided relevant information that allows an evaluation of the issues laid down in the aim of the Act, regarding ethical values, social justification of the GMO within a sustainable development. Given this lack of necessary information for such an evaluation, the Applicant has not demonstrated a benefit to the community and a contribution to sustainable development from the use of 1507 x 59122 x MON 810 x NK603. The Applicant should thereby provide the necessary data in order to conduct a thorough assessment on these issues, or the application should be refused.

It is also important to evaluate whether alternative options, (e.g. the parental non-GM version of 1507 x 59122 x MON 810 x NK603 has achieved the same outcomes in a safer and ethically justified way.

Further, the Norwegian Gene Technology Act, with its clauses on societal utility and sustainable development, comes into play with a view also to health and environmental effects in other countries, such as where GMOs are grown. For instance, it is difficult to extrapolate on hazards or risks taken from data generated under different ecological, biological, and genetic 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.

Recommendation: The Applicant should submit required information on the social utility of 1507 x 59122 x MON 810 x NK603 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

2.2 Ethical considerations

The event 1507 x 59122 x MON 810 x NK603 contain genes that confer specific resistance to glyphosate ammonium, a herbicide that is banned in Norway. The evaluation of co-products, that is, secondary products that are specifically designed and intended to be used in conjunction with the GMO, is 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, particularly when there is precedence in policy concerning its used independently.

While it is understood that the Applicant has not applied for deliberate release of 1507 x 59122 x MON 810 x NK603 in Norway, the acceptance of a product in which the intended use includes the use of a product banned in Norway would violate basic ethical and social utility criteria, as laid out in the Act. 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 on the other. This line of reasoning is consistent with the provisions under the Act to assess ethical, social utility and sustainable development criteria not only for Norway, but also for countries from which Norway imports food.

Therefore, we find it difficult to arrive at justified use of these events without engaging in such an ethical double standard. 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 favourable or unfavourable social consequences that may arise from the use of the genetically modified organism(s)", respectively.

1507 x 59122 x MON 810 x NK603 as a stand-alone products may prove to be perfectly as safe as its conventional counterpart, yet with consideration of co-product usage this can not be concluded on the basis of the information presented in this application.

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 LMO, 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 conceptual, empirical and informational deficiencies in the dossier that do not justify a conclusion of safe use, social utility and contribution to sustainable development of 1507 x 59122 x MON 810 x NK603. Assumption-based reasoning should not be substituted for scientific testing to bring greater certainty to the assessment and confidence in the results. 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. 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 1507 x 59122 x MON 810 x NK603, we conclude that based on the available data, including the safety data supplied, the Applicant has not substantiated claims of safety satisfactorily to warrant approval in Norway at this time.

References

Appenzeller, L.M., Malley, L., MacKenzie, S.A., Hoban, D., Delaney, B., (2009) Subchronic feeding study with genetically modified stacked-trait lepidopteran and coleopteran resistant (DAS-Ø15Ø7-1xDAS-59122-7) maize grain in Sprague-Dawley rats. *Food Chem. Toxicol.* 47:1512-20

Axelrad J C, Howard CV, Mclean WG, (2003) The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon. Toxicol 185:67-78.

BAT. Biosafety Assessment Tool (GenØk and University of Canterbury). www.bat.genok.org/bat.

Blackburm LG and Boutin C (2003) Subtle effects of herbicide use in the context of genetically modified crops: a case study with glyphosate (Roundup®) Ecotoxicol, 12:271-285

CBD (2003). Cartagena Protocol on Biosafety. http://www.cbd.int/biosafety/

Codex, 2003. Principles For The Risk Analysis Of Foods Derived From Modern Biotechnology; Codex Alimentarius Commission, CAC/GL 44-2003

Codex (2003a). Codex Work on Foods Derived from Biotechnology. In CAC/GL 45-2003. Codex. http://www.who.int/foodsafety/biotech/codex_taskforce/en/.

Codex (2003b) Codex Alimentarius Commission, Alinorm 03/34: Joint FAO/WHO Food Standard Programme, Codex Alimentarius Commission, Twenty-Fifth Session, Rome, Italy, 30 June- 5 July, 2003. Appendix III, Guideline for the conduct of food

safety assessment of foods derived from recombinant-DNA plants, and Appendix IV, Annex on the assessment of possible allergenicity, pp. 47-60

Dallegrave E, Mantese FD, Coelho RS, Pereira JD, Dalsenter PR, Langeloh A (2003). The teratogenic potential of the herbicide glyphosate-Roundup (R) in Wistar rats. Toxicol Lett 142:45-52.

Dolezel M, Miklau, M., Eckerstorfer, M., Hilbeck, A., Heissenberger, A., Gaugitsch, H., 2009. Standardising the Environmental Risk Assessment of Genetically Modified Plants in the EU / Standardisierung der Umweltrisikoabschätzung gentechnisch veränderter Pflanzen in der EU. BfN – pp. 259.

De Schrijver A, Devos Y, Van den Blucke M, Cadot P, De Loose M, Reheul D and Sneyer M (2006) Risk assessment of GM stacked events obtained from crosses between GM Events. Trends in Food and Sci Technol XX, 1-9.ONDde69

EFSA 2008 (EFSA-GMO-UK-2005-20

EFSA 2009c (EFSA-GMO-RX-NK603/ EFSA-GMO-NL-2005-22)

EFSA 2009d (EFSA-GMO-UK-2005-21)

EFSA (2010) EFSA Panel on Genetically Modified Organisms (GMO); Draft Scientific Opinion on theassessment of allergenicity of GM plants and microorganisms and derived food and feed. *The EFSA Journal* 8(7):1700

EFSA GMO Panel/Unit (EFSA/GMO/472, http://www.efsa.europa.eu/en/gmomsmeeting/docs/gmo090113no-m.pdf

EFSA Scientific Committee; Statistical Significance and Biological Relevance. EFSA Journal 2011; 9(9):2372. [17 pp.] doi:10.2903/j.efsa.2011.2372. Available online: www.efsa.europa.eu/efsajournal

Fagard M and Vaucheret H (2000) (Trans) Gene Silencing in Plants: How many mechanisms? Annu Rev Plant Physiol Plant Mol Biol, 51:167-94.

Fasoli, E., Pastorello, E.A., Farioli, L., Scibilia, J., Aldini, G., Carini, M., Marocco, A., Boschetti, E., Righetti, P.G. (2009) Searching for allergens in maize kernels via proteomic tools. *J. Proteomics* 72:501-10

Gardner MJ & Altman DG, 1986. Confidence intervals rather than P values: estimation rather than hypothesis testing. British Medical Journal, 292, 746-750.

Gomord, V., Chamberlain, P., Jefferis, R. & Faye, L. (2005). Biopharmaceutical production in plants: problems, solutions and opportunities. *Trends Biotechnol.* 23, 559-565. Elbers IJ W et al 2001

Guimaraes, V.D., Drumare, M-F., Ah-Leung, S., lereclus, D. Bernard, H. Creminon, C., Wal, J-M., Adel-Patient, K (2008) Comparative study of the adjuvanivity of Bacillus Thuringiensis Cry1Ab protein and cholera toxin on allergic sensitization and elicitation to peanut. Food and Agricultural Immunology, 19. 325-37.

Halpin C (2005) Gene stacking in transgenic plants- the challenge for 21st centry plant biotechnology. Plant Biotechnol, 3: 141-155.

He, X.Y., Huang, K.L., Li, X., Qin, W., Delaney, B., Luo, Y.B. (2008) Comparison of grain from corn rootworm resistant transgenic DAS-59122-7 maize with nontransgenic maize grain in a 90-day feeding study in Sprague-Dawley rats. *Food Chem. Toxicol.* 46 (6):1994-2002

Hung D, (2007) Diffused brain injury in glufosinate herbicide poisoning. Clinical Toxicol 45:617.

Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P (2003) Biochemical and histopathological effects of glyphosate herbicideon Nile tilapia (Oreochromis niloticus). Environ Toxicol 18:260-267.

Küster, B., Krogh, T.N., Mørtz, E. & Harvey, D.J. (2001). Glycosylation analysis of gel-separated proteins. *Proteomics* 1, 350-361.

Lee PN, Lovell D, 2009. Statistics for toxicology; General and Applied Toxicology, Third Edition, John Wiley & Sons, Ltd.

Marc J, Mulner-Lorillon O, Boulben S, Hureau D, Durand G, Belle R (2002) Pesticide roundup provokes cell division dysfunction at the level of CDK1/cyclin B activation. Chem Research Toxicol 15:326-331.

Matsumura N, Takeuchi C, Hishikawa K, Fujii T, Nakaki T (2001) Glufosinate ammonium induces convulsion through N-methyl-D-aspartate receptors in mice. Neuroscience Letters 304:123-125.

Martínez, C., Ibarra, J.E., Caballero, P. (2005) Association analysis between serotype, *cry* gene content, and toxicity to *Helicoverpa armigera* larvae among *Bacillus thuringiensis* isolates native to Spain. *J. Invert. Pathol.* 90:91–97

Mesnage R, Clair E, Gress S, Then C, Szekacs A and deraline G-E (2011) Cytotoxicity on human cells of Cry1AB and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. Appl Toxical 2012, Feb 15. doi: 10.1002/jat.2712. [Epub ahead of print]

OECD TG 451 Draft OECD Guideline for the testing of chemicals, Test Guideline 451: Carcinogenicity studies.

Ono MA, Itano EN, Mizuno LT, Mizuno EHF, Camargo Z P (2002) Inhibition of Paracoccidioides brasiliensis by pesticides: Is this a partial explanation for the difficulty in isolating this fungus from the soil? Medical Mycology 40:493-499.

Pastorello, E.A., Pompei, C., Pravettoni, V., Farioli, L., Calamari, A.M., Scibilia, J., Robino, A.M., Conti, A., Iametti, S., Fortunato, D., Bonomi, S., Ortolani, C. (2003) Lipid-transfer protein is the major maize allergen maintaining IgE-binding activity after cooking at 100 degrees C, as demonstrated in anaphylactic patients and patients with positive double-blind, placebo-controlled food challenge results. *J. Allergy Clin.Immunol.* 112(4):775-83

Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE (2005) Differential effects of glyphosate and roundup on human placental cells and aromatase. Environmental Health Perspectives 113:716-720.

Schulte-Hermann R, Wogan GN, Berry C, Brown NA, Czeizel A, Giavini E, Holmes LB, Kroes R, Nau H, Neubert D, Oesch F, Ott T, Pelkonen O, Robert-Gnansia E, Sullivan FM, (2006) Analysis of reproductive toxicity and classification of glufosinate-ammonium. Regulatory Toxicology and Pharmacology 44:S1-S76.

Solomon KR, Thompson DG (2003) Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. Journal of Toxicology and Environmental Health-Part B Critical Reviews 6:289-324.

Spök A, Gaugitsch H, Laffer S, Pauli G, Saito H, Sampson H , Sibanda E, Thomas E, van Hage M and Valenta R (2005) Suggestions for the assessment of the allergenic potential of genetically modified organisms. Int Arch Allergy Immunol, 137: 167-180.

Then C, (2009) Risk assessment of toxins derived from Bacillus thuringiensis – synergism, efficacy, and selectivity. Environ Sci Pollut Res DOI 10.1007/s11356-009-0208-3.

Walker, D.R., Walker, A.K., Wood, E.D., Bonet Talevera, M.E., Fernandez, F.E., Rowan, G.B., Moots, C.K., Leitz, R.A., Owen, P.A., Baxter, W.E., Head, J.L., Boerma, H.R. (2006) Gametic selection by glyphosate in soybean plants hemizygous for the CP4 EPSPS transgene. *Crop Sci.* 46:30–35

WHO (2006) GEMS/FOOD Consumption cluster diets, revealing per capita consumption of raw and semi-processed agricultural commodities in defined country clusters. Prepared by the Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food) of the World Health Organisation (WHO) (http://www.who.int/foodsafety/chem/gems/en/index1.html)

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