GenØk - Centre for Biosafety

Vår ref:2012/h107
Deres ref: 2012/6554 ART-BI-DHT

Assessment of the technical dossier submitted under EFSA/GMO/NL/2012/107 for approval of transgenic crop, MON810 pollen, Monsanto Company

Submitted to

Direktoratet for Naturforvaltning

By

Lise Nordgård og Idun Merete Grønsberg

Centre for Biosafety – GenØk
August 2012

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 MON810 pollen. Søker har ikke inkludert noe av den informasjonen omkring samfunnsnytten og bærekraftighet til MON810 pollen 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 som omfatter mat produsert fra MON810 pollen eller inneholdende ingredienser produsert fra MON810 pollen. I følge søker er maisen genmodifisert til å ha resistens mot enkelte arter i orden Lepidoptera.

Informasjonen som er tilgjengelig fra søker er ikke tilstrekkelig for uavhengig evaluering av søknaden. Det foreligger bl.a. ingen resultater fra analyser eller detaljerte forsøksoppsett til toksikologiske/immunologiske effekter eller foringsforsøk i relevante dyremodeller med MON810 pollen. Basert på manglende uavhengige studier og data tilgjengelig ønsker vi å påpeke at det er kunnskapshull relatert til risiko for helse og miljø ved MON810 pollen.

Søker gir ikke opplysninger som adresserer vurderingskriteriene bærekraft, samfunnsnytte og etiske aspekter som forutsettes anvendt i den norske genteknologiloven. I denne sammenheng er det viktig å få dokumentert erfaringer med hensyn på effekter på miljø, helse og samfunnsaspekter. Denne type dokumentasjon er ikke vedlagt søknaden om omsetting av mat produsert fra MON810 pollen eller inneholdende ingredienser produsert fra MON810 pollen.

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av MON810 pollen i de bruksområder det søkes om.

Konklusjonen er basert på

i) manglende dokumentasjon av helse og miljøeffekter med MON810 pollen
ii) bruken av føre-var prinsippet ved kunnskapshull og vitenskapelig usikkerhet.

SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED EFSA/GMO/NL/2012/107

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 MON810, 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.

Specific 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:

The regulators are encouraged to fill the research gaps when it comes to

- Studies using the plant version of the protein in these analyses to get the most authentic results
- Stuides with MON810 pollen derived proteins
- The regulator is encouraged to conduct a review of the environmental and health-related adverse effects to determine whether CryIAb meets satisfactory criteria for safety within the Norwegian regulatory context.

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 MON810 pollen. **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.**

Therefore, in our assessment of MON810 pollen, 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/2012/107

About the event

According to the developer, MON810 pollen has been genetically modified to express the Cry1Ab protein, derived from *Bacillus thuringiensis* subsp. *kurstaki*, which confers protection against predation by certain leidopteran insects pests, including the European Corn Borer. The particle acceleration transformation method was used in the development of MON810.

Assessment findings

The Applicant states that "MON810 is as safe as its conventional counterpart with respect to potential effects on human and animal health", and more specifically to pollen, that "the genetic modification in MON810 maize does not constitute an additional health risk if MON810 maize pollen were to replace maize pollen from non-GM maize in or as food". Further the applicant states that "Cry1Ab has been reviewed and considered safe in several occasions".

Impact of GMOs on beekeeping sector

One of the concerns when it comes to GM pollen is the possibility to maintain the possibilities of GMO-free honey production since the honey producers are not allowed to sell honey that contains pollen from MON810 for human consumption. This is particular true for beekeepers in the EU and beekeepers outside of the EU trying to export to Europe, where the customers are sensitive to the GMO issue. In its ruling, the European Court of Justice has decided that pollen is an "ingredient" in honey. Any food containing a GM ingredient is considered "produced from a GM organism" and therefore regulated, according to European rules which mean that honey containing GM pollen cannot be marketed without authorization (Regulation (EC) 1829/2003).

Pollen mixing with honey is nothing new. Bees store pollen in the hive as food for larvae, and small amounts of pollen from these storage areas are also mixed with honey when beekeepers harvest it (Waltz E, 2011). Honey bees cover a large foraging area of several square kilometers. When bees collect nectar, pollen, honeydew, resin and water, they cannot distinguish between conventional and genetically modified plants. Such an extremely open production system raises a very complex set of problems for the beekeeping sector and regulators (Haefker W). In field trials with genetically modified plants, visits by honey bees can contribute to the dispersal of pollen of such plants to areas outside the field trial area and if this pollen could end up in honey produced by local beekeepers, pollen dispersal by honey bees should be considered as a potential risk (Waltz E, 2011).

Beekeepers around the world have pointed out considerable deficits in assessing the implications of GMO cultivation for beekeeping and in maintaining the conditions for GMO-free honey production

Safety of the Cry1Ab protein for the environment and human health

The applicant states that "Cry1Ab has been reviewed and considered safe in several occasions". However, the Applicant fails to address the relevant literature on the environmental and health implications of the Cry I class of proteins, and Cry1Ab in particular.

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.
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 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 transgenic insecticidal protein Cry1Ab in aquatic environments and suggest that that sustained release of this potently bioactive compound from Bt maize production could result in negative impact on aquatic biodiversity. 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.

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.

Molecular characterization

The Applicant states that the data on molecular characterization did not identify features of maize MON810 pollen with a potential to raise any safety concerns. The Applicant claims that since the safety of the Cry1Ab protein in MON810 reached for food/feed has previously been assessed by the EFSA GMO panel it also apply to pollen.

Assessment of the newly expressed protein
Firstly, all data on the "newly expressed protein" is old data from previous dossiers on Mon810. The dossier does not mention if the Cry1Ab protein from pollen is analysed when it comes to developmental expression of the protein. It goes through expression levels in tissues of the maize plant that has been reported for previous applications of Mon810. As an example: table 5 in the Technical dossier (page 52) only considers leaf, whole plant, grain and overseason leaf. In the field trials performed in 1994 (US) and 1995 (Europe) pollen is also not analysed/measured/considered, although these data are used and presented in the present application of Mon810 pollen.

Next, the Cry1Ab toxin used by the applicant was isolated from a surrogate source (bacteria) rather than a commercial GM plant and structural equivalence between that produced in GM plants and that produced in bacteria was not demonstrated. The applicant uses *E.coli* produced Cry1Ab as the levels of introduced protein *in planta* is too low. This bacterial version was used for *in vitro* digestibility, acute oral toxicity and heat stability analysis of the protein.

The applicant provides evidence for equivalence between plant and bacterial version of Cry1Ab, data that has been shown previously for approval of Mon810. However, the applicant should search to use the plant version of the protein in these analyses to get the most authentic results as the two proteins are expressed in bacteria and plant. 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).

Allergenicity assessment
Allergenicity of the Cry1Ab protein is tested through Codex Alimentarius, 2003 (Codex, 2003). The assessment is heavily based on that the protein is from a non-allergenic source, has no structural similarities to known allergens and is rapidly digested. The conclusion from the applicant is that the protein is not allergenic. They do not discuss potential allergenicity of the plant derived version of the protein isolated from pollen itself, but rely on data obtained from the bacterial version.

The presence of fusion proteins is analysed. The data are from 2002-03 using bioinformatics tools for the analysis and also analysis using recent allergen and toxin databases for new analysis of the 3 flanking region. Both 5 and 3 juntions of the protein is analysed and the data suggests that there is no similarity to allergens, toxins or pharmaceutically active proteins

present. However, one peptide is discovered in the 3 junction that is mentioned specifically, but analysed further. The bioinformatic analysis takes into account continuous stretches of amino acids that can represent potential peptides working as epitopes in the immune system. However, it is also known that discontinuous epitopes, resulting from the tertial structure of proteins, also can act as triggers of the immune system. This is however not mentioned in this assessment of the allergenicity. The Cry1Ab protein is not considered to have an effect on human or animal health because of its previous evaluation of safety. This is to expect "the expected", and not the "unexpected". This is should be analysed further in the context of Mon810 pollen.

The applicant states that the low amount of protein (only 0.0004% of the protein expressed in the gene modified maize plant is Cry1Ab) does not represent allergenicity, However, even trace amounts of some allergens can be very potential triggers of the immune system.

Toxicological assessment
The assessment of potential toxicity of the expressed Cry1Ab protein is included in the dossier with the same arguments as previous GM plants with the same inserted gene. These arguments are the ones that are most commonly used: history of safe use, no structural similarity to known toxins, no acute toxicity effects to mammals and rapid digestion in digestive fluid. All studies are performed with the *E.coli* derived protein, and not the plant version of it, except in the feeding studies. ***There are however no studies performed with Mon 810 pollen derived proteins.***

Recommendaton:

- The applicant should search to use the plant version of the protein in these analyses to get the most authentic results as the two proteins are expressed in bacteria and plant.
- Stuides with MON810 pollen derived proteins should be included

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

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 MON810 pollen. 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 MON810 pollen 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 MON810 pollen and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

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 issues in the dossier that do not justify a conclusion of safe use, social utility and contribution to sustainable development of MON810 pollen. 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 MON810 pollen 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

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

Bøhn T, Primicerio R, Hessen DO, Traavik T (2008) Reduced fitness of *Daphnia magna* fed a Bt-transgenic maize variety. Arch Environ Contam Toxicol DOI 10.1007/s00244-008-9150-5.

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

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/.

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.

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

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

EC, Regulation 1829/2003.

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

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

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

Hilbeck A, Schmidt JEU (2006) Another view on Bt proteins - how specific are they and what else might they do? Biopestic. Int. 2:1-50.

Küster B, Krogh TN, Mørtz E & Harvey DJ (2001) Glycosylation analysis of gel-separated proteins. Proteomics 1:350-361.

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

Marvier M, McCreedy C, Regetz J, Kareiva P (2007) A Meta-Analysis of Effects of Bt Cotton and Maize on Nontarget Invertebrates. Science 316:1475-1477.

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

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

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

UMWELTBUNDESAMT (2009): Eckerstorfer, M., Heissenberger, A. & Gaugitsch, H.: SUPPLEMENTARY RISK ASSESSMENT FOR GM MAIZE MON 810 WITH REGARD TO THE CONCLUSIONS OF THE WTO-PANEL IN
THE CASE „EC BIOTECH" ON AUSTRIAN SAFEGUARD MEASURES FOR
GM MAIZE. Umweltbundesamt, Wien. ISBN 978-3-902611-07-9

Waltz E (2011) European ruling raises specter of mandatory GM pollen tests on honey. Nature biotechnol 29:958.