



GenØk - Centre for Biosafety

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**Assessment of the technical dossier submitted under
EFSA/GMO/DE/2011/95 for approval of transgenic mais event
5307 by Syngenta Crop Protection AG**

Submitted to

Direktoratet for Naturforvaltning

by

**Centre for Biosafety – GenØk
and
Center for Integrated Research in Biosafety
August 2011**

KONKLUSJON PÅ NORSK

Vi trekker frem flere begrepsmessige, empiriske og informatoriske mangler i dossieret som ikke gir grunnlag for en konklusjon om sikker bruk, samfunnsnytt og bidrag til bærekraftighet av 5307 mais. Søker har ikke fremskaffet noe av den informasjonen som er nødvendig for å kunne vurdere samfunnsnytt 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 samt at en ny søknad bare bør vurderes om søker har adressert de mangler vi har belyst.

SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL/2011/93

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 5307, 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 focused 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 not directly referenced refer to the document Part 1 of the technical dossier submitted by the Applicant.

Key findings

After a detailed analysis of many of the portions of the dossier on 5307 submitted by the Applicant, we outline a number of informational, methodological and conceptual weaknesses that do not justify the Applicant’s conclusion of safety, based on the given data. Our input focuses on a critique of the Applicant’s dossier and covers three broad issues:

1. Flawed assumptions, reasoning, or interpretations by the Applicant
2. Missing, incomplete or inadequate information to support the Applicants claims
3. Missing or incomplete information in relation to requirements under the Norwegian Gene Technology Act

Within each section we suggest a possible action that may help address the specific deficiencies where possible, and conclude our assessment with a summary recommendation.

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

Recommendations

Based on our findings, we propose a number of general recommendations, summarized here, with a few specific cases highlighted detailed in the critique below.

The Direktoratet for naturforvaltning is encouraged to request the following:

1. The Applicant should provide information on potential functional and structural changes that may have occurred in the production of the chimeric Cry3A-Cry1AB transgenic protein in 5307.
2. 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 \leq one target/tetraploid genome.
3. The Applicant should clarify 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.
4. The Direktoratet for naturforvaltning should request data from proper immunostimulation and allergenicity testing of 5307 including tests from diet and inhalation exposures.
5. The Applicant should provide evidence that the antibodies used in the protein characterization would detect all novel in-planta produced isoforms.
6. The Applicant should provide experimental data on protein specificity to substantiate claims of equivalence between the test protein and the in-planta produced form.
7. 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

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 5307. **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 5307, 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/DE/2011/95

About the event

According to the Applicant's dossier, the transgenic maize 5307 has been genetically engineered to express a chimeric *ecry3.1Ab* gene encoding the eCry3.1Ab protein, consisting of a protein derived from the native Cry3A protein from *B. thuringiensis* subsp. *tenebrionis*, and the Cry1Ab protein from *B. thuringiensis* subsp. *kurstaki* HD-1 for control of certain coleopteran pests. Further, a *pmi* gene, encoding the PMI protein as a selectable marker to utilize mannose as the primary carbon source under selective media.

1. Flawed assumptions, reasoning, or interpretations by the Applicant

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 on a modified Cry3A protein (mCry3A), derived from the Cry3A protein from *B. thuringiensis* subsp. *tenebrionis*, and on the Cry1Ab protein from *B. thuringiensis* subsp. *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.

<p>We recommend that the Direktoratet for naturforvaltning request information from the Applicant information related to potential functional and structural changes that may have occurred in the production of the chimeric Cry3A-Cry1AB transgenic protein in 5307.</p>
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2. Missing, incomplete or inadequate information to support the Applicants claims

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 sequences into 5307.

'Backbone' transfers are common when introducing recombinant DNA using the Ti plasmid system found in *Agrobacterium*. Historical data underestimates the number of backbone transfers because: "Usually, transfer of only the non-T-DNA sequences to the plant would remain undetected because: (1) there is no selection for the transfer of such sequences; and (2) scientists generally have not looked for the transfer of these sequences" (Kononov et al., 1997). The amount of DNA that can transfer can be many times the length of the T-DNA region: "extremely long regions of DNA (greater than 200 kbp) can transfer to and integrate into the genome of plants" (Kononov et al., 1997). Short backbone 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 material. Unfortunately, in this case only one probes (5312 bp) corresponding to the entire backbone sequence was used. Such large probes are prone to giving false negative results because 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 much smaller probes (www.bat.genok.org/bat).

Taking together the above problems in methodology and reporting, there is insufficient evidence to claim "5307 maize is free of backbone sequence from the transformation plasmid pSYN12274." (p. 56 technical dossier).

We recommend that the Direktoratet for naturforvaltning 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 \leq 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 in-planta 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.

3. Missing information in relation to requirements under the Norwegian Gene Technology Act

3.1. 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 5307. 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 exist that may achieve the same outcomes in a safer and more ethically justifiable 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.

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.

Conclusion

Available information for risk assessment evaluation

This evaluation is for the most part based on the Applicant’s own submitted information. The directly relevant scientific literature is very limited in some cases, yet we have tried to extract relevant indirect information from the peer-reviewed literature.

All 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 those data. The lack of compelling or complete scientific information to support the claims of the Applicant highlights the need for independent evaluation of safety studies and molecular information provided, including the raw data produced by the Applicant. We therefore request that mechanisms become elucidated that would allow any scientific information used in pursuit of regulatory approval to be transparent. 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 5307. 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 5307 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.

Kononov, M.E., Bassuner, B., Gelvin, S.B. (1997). Integration of T-DNA binary vector 'backbone' sequences into the tobacco genome: evidence for multiple complex patterns of integration. *Pl. J.* 11: 945-957.

Kuester, B., Krogh, T.N., Mortz, E., Harvey, D.J. (2001). Glycosylation analysis of gel-separated proteins. *Proteomics* 1(2): 350-361.