GenØk - Centre for Biosafety

Vår ref:2011/h77
Deres ref: 2011/2012 ART-BI-BRH

Assessment of the technical dossier submitted under EFSA/GMO/NL/2010/77 for approval of transgenic cotton event GHB614xLLCotton25 by Bayer Cropscience

Submitted to

Direktoratet for Naturforvaltning

by
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March 2011

Konklusjon på norsk

Basert på våre funn foreslår vi en rekke konkrete anbefalinger som vi adresserer i vårt høringssvar, og som vi har oppsummert her.

Direktoratet for Naturforvaltning oppfordres til å be om:

1. Søker må fremskaffe eksperimentelle bevis for fravær av kombinatoriske effekter som kan oppstå når man uttrykker to eller flere proteiner samme plante i stedet for å anta at denne kombinasjonen ikke er skadelig basert på vurderinger gjort av disse proteinene hver for seg.

2. Søker har valgt statistiske metoder for å analysere komparative data som bare gir en tolkning om likhet dratt på grunnlag av *ikke-signifikante* statistiske funn. Dette er ikke en konklusjon om trygghet dratt på grunnlag av statistisk signifikans i materialet, men faktisk det motsatte. Vi anbefaler at søker må gjenta de analysene som sammenligner GM og umodifiserte planter med metoder som er bedre egnet for å identifisere *statistiske signifikante* funn, f.eks. Bayesian metodikk eller alternativ hypotesetesting.

3. Vi mener at søker ikke har sammenlignet dataene fra GM planter med en egnet gruppe. Vi mener derfor at de sammenlignede analysene mellom GM og umodifiserte planter må gjentas og sammenlignes med kun den riktige kontroll gruppen (isogen linje). Når man finner forskjeller mellom gruppene, må søker følge opp viktigheten av disse funnene i stedet for å utføre flere analyser med irrelevante komparatorer.

4. Søker må fremskaffe informasjon som er påkrevd i Genteknologiloven om samfunnsnytten av GHB614 x LLCotton25 samt bidraget til bærekraftig utvikling (inklusive data om bruk av plantegifter i de land som dyrker denne GM-bomulls varianten).

Hovedkonklusjon og anbefalinger

Vi har i vår gjennomgang funnet flere svakheter av begrepsmessig art, mangel på informasjon, feilaktige konklusjoner og mangelfulle empiriske data som hver for seg og til sammen ikke støtter søkers påstand om sikker bruk av GHB614xLLCotton25. Søker har 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 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/2010/77

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 GHB614xLLCotton25, 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 "Glyphosate and glufosinate ammonium-tolerant GM cotton GHB614 x LLCotton25 for food and feed uses, and for import and processing" submitted by the Applicant.

Key findings

After a detailed analysis of many of the portions of the dossier on GHB614 x LLCotton25 submitted by the Applicant, we outline a number of informational, methodological and conceptual weaknesses that do not justify the Applicant 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. Faulty assumptions, reasoning, or interpretations by the applicant

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

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

Within we suggest appropriate action to 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 this 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:

1. The Applicant should provide direct evidence of the lack of combinatorial effects arising from the expression of the two target proteins in one plant, instead on relying on the assessment of non-harm of the target genes existing independently, before a conclusion of safety can be scientifically justified.

2. The Applicant has chosen statistical methods for interpreting comparative data that allow only an inference of similarity from *non-statistically signficant* findings between comparative groups. We recommend the applicant should reanalyze the data using methods (Bayeian methods, alternative hypothesis testing) more appropropriate for identifying *statistically significant* differences.

3. The Applicant should re-analyse the comparative assessment using only the appropriate (isogenic) comparators and baselines. When differences are found, the Applicant should follow up on the significance of the observation, rather than perform post-hoc analysis using irrelevant comparators.

4. The Applicant should submit required information on the social utility of GHB614 x LLCotton25 and its contribution to sustainable development (including data on herbicide usage in cultivation countries) in accordance with the Norwegian Gene Technology Act.

Overall recommendation

The inferential, empirical and informational deficiencies identified in the dossier do not support claims of safe use, social utility and contribution to sustainable development of GHB614 x LLCotton25 . **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 GHB614 x LLCotton25, 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/2011/77

About the event

The transgenic cotton event GHB614 x LLCotton25 , developed by Bayer CropScience, has been genetically engineered for the expression of a modified EPSPS (2mepsps) gene conferring resistance to glyphosate-based herbicides, and a *bar* coding sequence with confers resistance to glufosinate ammonium based herbicides. The event was produced by breeding of the two transgenic lines GHB614 and LLCotton25, resulting the "stacked" event GHB614 x LLCotton25 .

Assessment

After a detailed analysis of many of the portions of the dossier on GHB614 x LLCotton25 submitted by the Applicant, we outline a number of informational, methodological and conceptual weaknesses that do not justify the Applicant conclusion of safety, based on the given data.

1. Faulty assumptions, reasoning, or interpretations by the applicant

1.1 Lack of consideration on possible and assessment of combinatorial effects arising from the inclusion of two ("stacked") target transgenic proteins

Throughout the dossier, the Applicant rationalizes the exclusion of event specific information on event GHB614 x LLCotton25 from an assumption of implied non-harm from parent plants (GHB614 and LLCotton25) already assessed for non-harm in prior submitted dossiers to the EU.

For example, the applicant states:

"The 2mEPSPS and PAT protein contents in the GTxLL cotton are similar to the protein contents of the single parents, GHB614 and LLCotton25. Consequently the combination of the two GM parents does not alter the expression of the inserted genes and therefore there is no interaction between the inserts." (p. 10)

and

"GTxLL cotton was developed by conventional crossing of the GHB614 and LLCotton25 lines. No new genetic modification was introduced in GTxLL cotton and therefore, there are no newly expressed proteins in GTxLL cotton other than the ones already assessed as safe in the case of GHB614 and LLCotton25." (p.84)

As such, assumptions of non-interaction by the Applicant are not supported by actual empirical evidence. Combinatorial effects, both beneficial and deleterious, may arise from the presence of a combination of synthetic genes that do not manifest from their presence separately. For instance, Heinemann and co-workers (2000) report a situation where a new phenotype arises from the combination of alleles *rpoB87* (rifampicin resistance) and *gyrA87* (nalidixic acid resistance), to confer protection against a third microbial agent, mitomycin-C. In *Arabidopsis* (plants), a combination of different alleles of *zwi-3* and *suz1* in the ZWI gene led to a male sterile phenotype, where no phenotype was observed when the two alleles were separated (Krishnakumar, S. & Oppenheimer, D. G., 1999).

Hence, the significant uncertainty surrounding the interactions of the stacked traits to produce tertiary phenotypes of biological or ecological relevance warrant direct evidence of lack of combinatorial effects when multiple target transgenic genes are introduced in one event. Thus, the statement by the Applicant "the combination of the two GM parents does not alter the expression of the inserted genes and therefore there is no interaction between the inserts" has not empirical basis and should be directly evaluated.

Recommendation: The Applicant should provide direct evidence of the lack of combinatorial effects arising from the expression of the two target proteins in one plant, instead on relying on the assessment of non-harm of the target genes existing independently, before a conclusion of safety can be scientifically justified.

1.2 Statistical inference of safety from non-significant findings of equivalence in comparative assessments

The Applicant performs a number of comparative assessments of plant composition, gene expression and agronomic performance of GMOs and their conventional counterparts. Within, the Applicant claims that no difference – and hence safety—can be deduced from non-statistically significant findings of equivalence in their assays.

For example, the Applicant states:

"Equivalence has been demonstrated between the GTxLL combined events in comparison with the conventional counterpart, cotton variety FiberMax 958, and the GHB614 and LLCotton25 parental events, by evaluation of the parameters defined by the cotton Plant Variety Protection Act. All the measured phenotypic parameters were inside the range for commonly cultivated cotton. No evidence of an unintended effect related to the GTxLL conventional cross has been indicated." (p.10).

However, statisticians have cautioned against the error of of inferring "sameness" from non-

significant statistical findings, (Brosi et al, 2009). Other approaches to evaluating comparative data may yield more prudent outcomes for where policy decisions may be involed (Taylor and Dizon 1996; Palsbøll et al. 2006). Approaches such as Bayesian methods (Wade 2000) and alternative hypothesis testing (McGarvey, 2007) represent promising yet wholly underutilized statistical methods for data used in a regulatory context.

One significant limitation in interpreting non-significant tests arises in situations where small samples sizes are used (as in the comparative tests performed by the applicant), as in these cases it is extremely difficult to find statistically significant differences between two groups (due to low power). Further, with small *effects sizes* (as may be encountered here), large samples sizes are required to observe a significant difference between populations. As such, alternative statistical methods used where both effect sizes and sample sizes are small, should be encouraged.

Recommendation: The Applicant has chosen statistical methods for interpreting comparative data that allow only an inference of similarity from *non-statistically signficant* findings between comparative groups. We recommend the applicant should reanalyze the data using methods (Bayeian methods, alternative hypothesis testing) more appropropriate for identifying *statistically significant* differences.

1.3 Choice of comparators and baselines

The selection of comparators by the Applicant deviates from the intended comparative approach in risk assessment, to analyse differences between the event under regulatory consideration with its parental conventional counterpart.

The Applicant states:

”As described in Section D.7.1, a comparative assessment between the GTxLL cotton and the commercially available variety FiberMax 958 with the same genetic background has been carried out. In addition, composition data derived from the GHB614 and LLCotton25 parental lines, grown in the same field trial as GTxLL, as well as from public available literature references, including data from non-GM cotton varieties, have been used as the baseline in the comparison with GTxLL cotton (Oberdörfer, 2009). ” (p. 56)

and

”Part of the substantial equivalence evaluation uses a comparison with reference ranges to look for consistent differences as an indication of unintended effects. The reference ranges from literature for cotton in commerce are included in the data sets to provide an expected range for each of the nutrients.” (p.58)

and

”Statistically significant differences in the comparisons between GTxLL (conventionally treated and treated with the test herbicides) and its non-transgenic comparator, variety FM 958, were found for ash, calcium, free and total gossypol, and dihydrosterculic acid. However, the mean values for these compounds calculated for the transgenic and non-transgenic groups are all within the references ranges for commercial cotton seeds (Tables 12 and 15).” (p.59)

As discussed, the extension of the concept of comparative assessment to include data on all cotton varieties, grown and measured in entirely different contexts, defies statistical logic and scientific robustness. This approach leads to underestimations of potentially signficant differences, some of which may be of biological relevance.

Recommendation: The Applicant should re-analyse the comparative assessment using only the appropriate (isogenic) comparators and baselines. When differences are found, the Applicant should follow up on the significance of the observation, rather than perform post-hoc analysis using irrelevant comparators.

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

The flawed reasoning and interpretations identified in Section 1 indicate that certain types of information required to determine the safe use of GHB614 x LLCotton25 will be lacking. *Please refer to Section 1 recommendations.*

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 (including regions where the GMO is grown). 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 GHB614 x LLCotton25. The Applicant should thereby provide the necessary data in order to conduct a thorough assessment on these issues, or the application should be considered

incomplete.

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. The Applicant, however, as not included information relevant information on how the event affects agricultural practices, particularly herbicide usage in conjunction with the added transgenic trait, in the countries where it is being grown. The Applicant has stated where the cotton is currently permitted for cultivation yet fails to include information related to herbicide use in these regions, and more information would be required to verify that a reduced usage in herbicide was leading to a environmental benefit when the event in question was used.

Lastly, it is also important to evaluate whether alternative options exist (e.g. the parental non-GM version of this GHB614 x LLCotton25) that can achieve the same outcome in a safer and more sustainable fashion.

Recommendation: The Applicant should submit required information on the social utility of GHB614 x LLCotton25 and its contribution to sustainable development (including data on herbicide usage in cultivation countries) 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 scientific information to support the claims of the Applicant highlights the need for independent evaluation of safety studies and molecular information. We therefore request that mechanisms become available that allow to all information, including any annexes that explain confidentiality claims invoked for some of the application information that may be of scientific relevance. Such independent evaluation is essential to maintaining rigorous standards expected in scientific practice. Despite the deficiencies in the dossier under examination here, we encourage the authorities to insist on this level of transparency and accessibility to raw data be considered where it is lacking.

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 GHB614 x LLCotton25. **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 GHB614 x LLCotton25 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

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