GenØk - Centre for Biosafety

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Assessment of the technical dossier submitted under EFSA/GMO/NL/2011/96 for approval of transgenic cotton, GHB119 from Bayer CropScience AG

Submitted to

Direktoratet for Naturforvaltning

by

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SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL/2011/96

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 GHB119, 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 "Insect resistant and glufosinate tolerant cotton event GHB119 for food and feed uses, and for import and processing in accordance with articles 5 and 17 of Regulation 1829/2003 GM Food and GM Feed", submitted by the Applicant.

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.

Specific recommendations

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

The Direktoratet for naturforvaltning is encouraged to request the following:

1. The Applicant should undertake safety assessments using versions of the target proteins derived from the plant variety/event under assessment.

2. The Applicant should submit required information on the social utility of GHB119 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

3. The Applicant should undertake safety assessments using versions of the target proteins derived from the plant variety/event under assessment.

4. The applicant should follow up short-term acute studies with longer term toxicity studies commensurate with the life cycle of the tested organism.

5. The Applicant should submit required information on the social utility of GHB119 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

Overall recommendation

Based on our assessment, we find that the deficiencies in the dossier do not support claims of safe use, social utility and contribution to sustainable development of GHB119. **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 GHB119, 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/2011/96

About the event

The transgenic GHB119, developed by Bayer CropScience AG, was developed via Agrobacterium-mediated transformation to confer insect tolerance through the expression of the Cry2Ae lepidopteran insecticidal toxin derived from the soil bacterium, *Bacillus thuringiensis*. GHB119 also contains the *bar* coding sequence encoding the specific enzyme phosphinothricin acetyl-transferase (PAT), that acetylates the herbicide glufosinate ammonium and thereby detoxifies the herbicide.

Assessment findings

1. Safety assessment of newly expressed proteins

The Applicant claims that Cry2Ae protein is not allergic or toxic to humans or animals. Again, the fact that the protein is expressed in a new host with a totally different translation and PTM machinery and the potential creation of new protein complexes, potential new proteins (recombinations) and possible biological responses to that are not discussed further. In the recent years, the adjuvancy of the Cry proteins has been of debate and also been addressed to the EFSA GMO Panel/Unit (EFSA/GMO/472). This has been seen in several animal experiments (Vasquez-Padron et al 1999, Guerrero and Moreno-Fierros 2007, Moreno-Fierros et al 2003.) and the mechanism(s) for that is under discussion. A report by Guimaraes et al. (2008) demonstrates the possible adjuvant properties of one of the Cry proteins (Cry1Ab) on the elicitation of the allergic reaction in the mouse model used. This is an import issue that should be considered further.

Recommendation: The Applicant should undertake safety assessments using versions of the target proteins derived from the plant variety/event under assessment.

2. In vitro digestibility in simulated gastric and intestinal fluids

While the applicant claims that Cry2Ae protein is reported to be rapidly degraded under gastric and intestinal simulated conditions, the proteins used in these analyses were derived from *E.coli* and not from the GHB119 plant itself. These results “confirm the safety or Cry2Ae and PAT for human and animal consumption due to the rapid degradation and minimizes the likelihood that these proteins can survive and be adsorbed thus eliciting a toxic or allergenic reaction”. It is suggested by FAO/WHO to include” [b]oth known non-allergenic (soybean lipoxygenase, potato acid phosphatase or equivalent) and allergenic (milk beta lactoglobulin, soybean trypsin inhibitor or equivalent) food proteins as comparators to

determine the relative degree of the expressed proteins pepsin resistance" (FAO/WHO, 2001, p.12). For the analysis of PAT, they have included HRP and OVA as reference proteins. An important study compared pepsin-catalyzed hydrolysis of proteins at pHs ranging from 2.0 to 4.0 and found that, at the pH of the infant stomach, antigenic epitopes were much more likely to survive and potentially pass into circulation (Schmidt, D.G. et al., 1995), however the Applicant makes not followup on the significance of this observation. pH stability is not evaluated here.

3. Acute toxicity tests

E.coli produced proteins (Cry2Ae >93% pure, PAT>90% pure) were used in animal studies (Rouquie 2006; Hêrouet et al 2005). The animals were observed for 15 days for clinical signs. No treatment related effects were observed. No clinical signs observed. No toxic or mortalities were observed for either of the two treatments. The proteins are thus claimed not to be toxic and that they are safe for animal and human consumption. In this assay they have used bacterially derived protein for their analysis. The aim must be to test the protein from its original source, cotton GHB119. In the feeding study, the proteins analyzed are administered to the mice only once, and it is not mentioned in the material and methods part whether they take blood samples before the study is started or during the experimental period. Many important biological parameters and reactions can change during a time-span of 15 days when the animals were observed. Induced reactions can normalize during that time period. Also, one should aim at testing the protein from the plant at different growth stages and of pollen to test for allergy reactions. Unforeseen interactions between plant derived proteins in the plant vs. the *E.coli* derived version is not considered here (potential interactions that can affect immunoreactivity etc).

Recommendation: The Applicant should undertake safety assessments using versions of the target proteins derived from the plant variety/event under assessment.

3.1 28 day toxicity study in mice with Cry2Ae protein

The Cry2Ae protein used in the toxicity study is isolated from *Bacillus Thuringiensis.* Animals were observed for 15 days for clinical symptoms. No treatment related changes were observed in any organs analyzed (Kennel 2011). Based on this work it is concluded that the protein is safe for human and animal consumption. It is questionable if organ changes can be observed after 28 days only. That would depend on what one is looking for. There is a proposed recommendation to EFSA that a **feeding** study should be for 1 year or more to look for cancer and/or toxicity related effects (OECD TG 451).

Recommendation: The applicant should follow up short-term acute studies with longer term toxicity studies commensurate with the life cycle of the tested organism.

4. Sequence homology with known toxins and allergens

Bioinformatics analysis conducted by the applicant (in silico approaches) indicated that the PAT protein has no homology to toxins, allergens or antinutrients (Capt 2010a, Capt 2011a) or that there is homology to known epitopes of allergens. The same is the case for the Cry2Ae protein (Capt, 2011b). This kind of analysis is widely accepted, but also requires additional testing (EFSA, 2008a; Gendel, S.M., 2002). The predictive value of the data obtained through bioinformatics is discussable as the incidence of allergy is low. Although a homology to a known toxin or allergen is not found based on sequence analysis, the protein folding and creation of new structures similar to known toxins/allergens should also be considered. The possibility that allergens can be predicted based on non-contiguous stretches of amino acids should also be paid attention to (Gendel, S.M 2002; Moreau, V. et al. 2006). The limitation of the in silico approach must be considered, as it is limited to identified proteins and epitopes that are not influenced by PTM (Codex 2003).

5. Testing of whole GM food/feed

No further testing of whole GM food/feed were considered necessary based on the results of the evaluations made in the application; a) substantial equivalence to conventional cotton is demonstrated, b) no concerns raised in toxicology evaluation of proteins produced in *E.coli*, c) equivalence of GHB119 and bacterially produced proteins demonstrated, and d) no other proteins of RNAs are expressed (no genetic transformation), (Technical Dossier p. 120). However, a feeding study with male broiler chickens was performed to supplement the safety evaluation (Stafford, 2009). This is in context with EFSA (EFSA, 2008b, .p43) saying that "in the context of genetic modifications involving the transfer of multiple genes, the potential risks of possible interactions between the expressed proteins, new metabolites and original plant constituents should be assessed".

No adverse effect on feeding, growth or general health were found of the GHB119 event or the gene insertion process itself. The number of chickens used in the study is high. However, the use of this type of experimental "animals" in feeding studies is somehow controversial as their health related problems will come as an addition to the potential problems that are posed on the animals in a feeding trial going over a long period. Also, the already health related problems might interfere with the interpretation of the results in the study. Only 21 of the animals from each gender/treatment group were analyzed although the number of animals in the beginning of the study is high. No adverse effects were detected in survival, body weight gain, feed consumption, feed conversion ratio, or in weight of chilled carcass, abdominal fat pad, legs, thighs, wings or breasts. However, it is unclear whether potential changes in the state of the internal organs were analyzed.

A long-term 90 day toxicity study in rats was also performed, where GHB119 cottonseed toasted meal (5-10%) was incorporated into the rat feed. Here, no biologically relevant changes were detected over a 13 week period (Totis 2010), although many organs and tissues were analysed for abnormalities. Also, no toxic or allergic effects from handling of GHB119 have been observed on field workers since first year of release (2002) (Technical Dossier p 121) however no supporting references are provided.

6. 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 GHB119. 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 GHB119) may achieve 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 GHB119 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

7. Ethical considerations in the use of the herbicide glufosinate as a co-product with GHB119 cotton

The intended co-product with this event is the herbicide glufosinate ammonium. Glufosinat ammonium is not legal for use 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 glufosinat ammonium is used (Hung 2007; Matsumura et al. 2001; Schulte-Hermann et al. 2006; Watanabe and Sano 1998). According to EFSA, the use of glufosinate ammonium will lead to exposures that exceed acceptable exposure levels during application.

The evaluation of co-products, that is, secondary products that are specifically designed and intended for use 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 GHB119 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.

GHB119 as a stand-alone product may prove to be perfectly as safe as its conventional counterpart, yet with consideration of co-product usage this cannot 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 issues in relation to the questionable safe use of GHB119 that do not justify a conclusion of safe use, social utility and contribution to sustainable development. 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 GHB119 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.

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