



GenØk - Centre for Biosafety

Vår ref:2012/h87
Deres ref: 2011/17087 ART-BI-BRH

Direktoratet for naturforvaltning
Tungasletta 2
7485 Trondheim
Dato: 01.02.2012

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet om høringer EFSA/GMO/NL/2010/87 for GT73 fra Monsanto Company.

Hvis du har noe spørsmål, vennligst ta kontakt.

Med vennlig hilsen,

David Quist

Forsker
GenØk – Senter for Biosikkerhet
david.quist@uit.no

Bidragstere:

Dr. Idun Merete Grønsberg

Forsker
GenØk – Senter for Biosikkerhet

Dr. Lise Norgård

Forsker
GenØk – Senter for Biosikkerhet

Marek Cuhra

Doktorgrad student
GenØk – Senter for Biosikkerhet



GenØk - Centre for Biosafety

Vår ref:2012/h87
Deres ref: 2011/17087 ART-BI-BRH

**Assessment of the technical dossier submitted under
EFSA/GMO/NL/2010/87 for approval of transgenic rapeseed,
GT73 from Monsanto Company**

Submitted to

Direktoratet for Naturforvaltning

by

**Centre for Biosafety – GenØk
February 2012**

SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL/2010/87

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 GT73 rapeseed, 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 authorization to place on the market GT73 oilseed rape in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed”, submitted by the Applicant.

Key findings

After a analysis of many of the portions of the dossier on GT73 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.

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 GT73. **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 GT73, 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/2010/87

About the event

According to the developer, GT73 has been genetically engineered to contain “one intact copy of the *goxv247* and *cp4 epsps* expression cassettes encoding the GOXv247 and CP4 EPSPS proteins, which confer tolerance to glyphosate”. (p.39)

Assessment findings

1. Information on toxic and allergenic effects

The data provided in the dossier do not give sufficient evidence that the use of GT73 oilseed rape is safe in relation to toxicology or allergenicity.

In the dossier presented by the applicant, acute toxicity studies in mice are presented to confirm the lack of mammalian toxicity of GOXv247 and CP4 EPSPS proteins. A number of issues create uncertainty regarding the claim of safety. First, acute toxicity was tested with *E.coli* purified GOXv247 and *E.coli* purified CP4 EPSPS protein which means that the study is using the bacterial version of the protein and not the plant-produced proteins, which exclude information on the toxicological potential of the protein in a genetically modified plant. One should always utilize the version present in the plant. Although one can prove that a protein from a plant and a bacteria is similar, it should be given specific attention to the choice of expression host, being it eukaryotic versus prokaryotic systems) since PTMs differ and may have an impact on the potential of allergenicity of a protein (Codex work on Foods derived from Biotechnology, CAC/GL 44-2003, p. 14 and 22).

Second, acute oral toxicity studies may detect large effects, yet have little relevance for substances or products which will be fed or consumed over a lifelong period and exhibit chronic effects. Certain toxicological properties will only become evident in case of systematic testing (Spøk et al 2004, 2005). With the acute toxicity study, mice and rats are the normal test organisms, but they should also include one non-rodent species (e.g. dogs) for sub-chronic testing. Proper hazard characterization of any effects noted in these studies may require determining mode of action (EFSA, 2008a).

Further the applicant refers to feeding studies with rats; a four weeks feeding experiments with rats fed oilseed rape seed from GT73 and GT200 (1994) a second experiment in 1995 and a third in 1996 on the background on variable liver weights in exp 1 and 2. In the end they conclude that liver weight increase observed in the 2. feeding study is related to normal variability without relevant histological examinations of the relevant organ and tissue. In all

the studies with rats only 10 animals/group were used, which would have too low statistical power to generate meaningful results.

Another study presented in the dossier is using broiler chickens (chickens raised for meat production) as animal model (Tayler et al 2004). The study design includes a high number of animals and reaches a great statistical power, however the use of broiler chickens in general is controversial since the animals present high mortality and a diverse and severe pathological conditions as it is. Other feeding studies were conducted in swine, trout, quail and lamb all concentrating on the growing potential of the different diets and comparing the weight of different parts of the animal (Mawson P et al 1994, Aalhus et al 2003). 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 GT73 (Spøk et al 2004).

Also it is significant that none of the diets given to the animals in any of the described feeding study presented any level of glyphosate, and since herbicide treated products will be the product humans and animals will be exposed to it should be taken into account in feeding trials to mimic real-world feed conditions.

2. Outcrossing potential

There is a detailed section on the potential for outcrossing, hybridization through drift of pollen from plant resulting from GT73 seed accidentally entering the existing agriculture systems. The application presents *B. napus* as primarily self-pollinating with a limited range of pollen drift through wind and insect pollinators. These spatial limitations for horizontal gene flow and hybridization with local varieties are considered sufficient to ensure the safety of the existing European cultivation of *B. napus*. However, several questions remain unresolved, one of them survivability and dissemination of GT73 seed from accidental spills. The applicant specifically states that *“Oilseed rape dissemination can occur by means of seeds and pollen. The seeds have no special or specific adaptations to facilitate widespread dispersal (they are not wind transported and have no structures to allow them to stick to animal fur) and so any shattered seed will remain in close proximity to the site of production. Further dissemination may occur by means of fauna or machinery.”* (p. 27, section 4a)

These statements are not elaborated further by the applicant. Although in the next section the applicant makes a contradictory claim commenting on the issue of “special factors affecting dissemination” stating that *“Seed dissemination is increased by excessive pod shattering during harvesting, but seed remains in the area where it is shed”*. (p. 27, section 4b).

This claim is unsubstantiated and not in accordance with well established scientific knowledge of seed dispersal mediated by birds and other organisms (Howe and Smallwood 1982). Continued traditional agriculture coexistence and implications for such coexistence from avifauna dispersal of viable commercial seed from genetically modified crops has recently been studied in transgenic crops in general (Cummings et al 2008) and in canola *Brassica napus* specifically (Twiggs et al 2008). Potential dispersal of viable seed by such vectors should not be underestimated and has to be addressed by the applicant.

Although Twigg et al 2008 estimates the dispersal range of viable *B. napus* seed as generally less than 10 kilometres in the species of birds investigated (wood duck, *Chenonetta jubata*), other observations indicate that the potential dispersal range could be much larger in other bird species. Evidence presented by Hart (2011) indicates that transgenic glyphosate tolerant *B. napus* originating from Canada, is dispersed into the USA by migrating geese. The issue has only been realized recently and mainly since the geese-mediated volunteers of *B. napus* turn up as resistant weed in agriculture of other glyphosate tolerant transgenic plants, in this specific case transgenic GT sugar beet in North Dakota.

3. Potential toxic compounds and allergenic agents, and feeding studies

The applicant describes two categories of naturally occurring toxicants, euric acid and glucosinolates, but does not give information on possible effects of the main issue concerning herbicide tolerant crops, namely the potential effects on health and environment from the substantial use of glyphosate, this herbicide being an unavoidable and integrated element of the cultivation of these varieties (p 28, section 7). Such potential effects of glyphosate use are not only restricted to the environment and ecosystem where the glyphosate-tolerant varieties are grown, but also has the potential to affect nutrient composition of the crops (Zobiolo et al 2010, 2011) as well as inducing substantial levels of pesticide residues and metabolites of pesticides in seed (Duke et al 2003, 2005. Cuhra and Bøhn in prep) thus influencing the quality of the produce.

In section 7.9.2 (p 137) the applicant is asked to describe the work done to assess the potential allergenicity of the GT73 variety. The applicant states that "*Oilseed rape is not known to be allergenic. GT73 has been demonstrated to be substantially equivalent to conventional oilseed rape, except for the GOXv247 and CP4EPSPS proteins, which have been demonstrated not to have characteristics of an allergen. Therefore, GT73 is considered not to have allergenic potential*". This claim is unsubstantiated by neither testing nor experimental evidence and based solely on the previously mentioned assumptions. The applicant has not demonstrated the GT73 variety to be substantially equivalent to conventional oilseed rape but merely claims this to be the case.

In a similar way, the conclusion presented by the applicant in section 7.10 f (page 147) regarding potential adverse effects on human health, is not based on adequate scientific evidence to conclude safety. The safety assessment conducted by the applicant only includes simple animal feeding studies on nutritional quality, with no histological examinations of relevant organs and tissue.

4. Environmental monitoring

Finally it must be mentioned that the applicant does not provide the environmental monitoring plan requested in the 2011 application (section 11, p 150). Such an environmental monitoring plan should also take into consideration potential environmental consequences of spillage during import, transportation, storage, handling and processing of GT73 within the area of the European Union. The applicant maintains that this monitoring plan is defined in the 2005 Commission Decision 2005/635/EC on import and handling of GT73 and thus not relevant in this new application concerning only the actual use of the commodities produced from GT73

material. Such avoidance on the part of the applicant should not be accepted by the regulator, especially as the mentioned Commission Decision (EU 2005) does not specify the extent and details of the monitoring plan. The environmental monitoring plan has high relevance and must ensure future coexistence of local European varieties of *B. napus* and related species potentially subject to contamination from the transgenic varieties. Even if the local varieties currently under cultivation may not reflect the original diversity of *Brassica* in the European centre of origin, the varieties are an important traditional part of agriculture in Central and Northern Europe.

5. Information on the sequences actually inserted or deleted: Southern analysis

Initial comments on southern blots and PCRs performed to verify insert integrity etc.: The sensitivity of the probes used in the southern(s) together with the stringency used during washes should be known as this affects the binding between the probe and the recombinant DNA in question. If southern blot is used properly, one can detect an insert of 15 bp (Svitashev SK et al 02). However, if the probes are too long, this can lead to false negative results and thus a failure to detect a potential insertion. This is caused by the long probe binding to a short recombinant sequence and the stringency of the wash that can wash the probe off its target. Many of the probes used in the Southern blots performed to verify the insert in the GT73 oilseed rape are very long (most of them are over 1 kb), together with the fact that the stringency of the performed washes is unclear. Thus, there is a potential that possible targets in the sequence analysed is missed/not detected. The stringency and the sensitivity of the blots for each probe used should be reported, also because the probe sizes vary considerably. The size of probes, wash stringencies and also exposure times can have an impact on the actual detection level of additional bands, that might give an indication of the behaviour and condition of the transgene insertion (Makarevitch et al 03).

In the report by Dobert R (1998) they have checked down to 7 pg of genomic DNA, corresponding to 0.5 copies of the vector backbone. But they have only performed southern detection with these amounts of DNA for two of the probes used.

In a report headed to the Norwegian Directorate for Nature Management (DN) by a commission in the Ministry of Environment (MD) (Report from DN, 280508, application C/NL/98/11, pp6) it was pointed out a need for more analysis of new open reading frames (ORFs). This was accomplished in a study performed in 2011 (Tu and Silvanovich).

Another issue is the presence or absence of backbone vector sequence in the line GT73. The two probes used in the southern are very long; 2703 and 2756 bp (Dobert R et al 98), and with the challenges connected to long probes, this should have been repeated with shorter probes to be able to detect potential false negative signals.

In general, the southern membranes provided in the dossier and also some of its reports lack labelled markers. How can you know that the band has the expected size without using a marker? Only two of the membranes have a marker present and these are the only nice blots presented (Figure 11-12 ,p 54-55, Technical Dossier Part I).

The data provided for the PCR and sequencing are old (Palmer 2003, Petersen 2000). There has not been an attempt to try to sequence the unexpected sized PCR products present in the analyses.

In the dossier, data on detection limits of both PCR and southern blot are unclear/not found or reported.

a) The copy number of all detectable inserts, both complete and partial

Determination of copy number was obtained using southern blot and PCR. Genomic DNA was extracted; DNA was RE cut and subjected to southern blot. The conventional line Westar was used as negative control and to determine non-specific dots on the blots and PV-BNGT04 used as positive control. The number of inserts were analysed by southern blot only, and it was detected a hmw (high molecular weight) band in all lanes (including the negative control Westar), and also an additional single hmw band in GT73, concluding that there only is one T-DNA inserted at the integration site. This analysis was done by using the whole PV-BNGT04 labelled plasmid as probe. No PCRs or sequencing reactions were performed in this specific analysis.

Copy number determination was analysed using both PCR and southern blot. Single copy and two copies (head to head, tail to tail and head to tail) were analysed for. The conclusion of the analysis is that there is only one copy of the insert present at a single insertion site in the GT73 genome. Of critics here is the size of probes used in southern, where 5 of 6 probes are >500 bp. Also, there is no overview on the stringency washes that has been used during southern blot. One of the membranes are badly labelled (Figure 7, pp 48) and several of them lacks a good size marker. For the matter of the gox247 and cp4 EPSPS cassette integrity analysed by southern, the additional bands detected for the PV-BNGT04 is explained as incomplete digestion or other forms of the plasmid (nicked, supercoiled, linearized etc.). However, the incomplete digestion is not analysed further.

Absence of backbone sequence from vector is also analysed by southern. Here, the membrane is bad, detection level is not mentioned, stringency wash conditions not mentioned, labelled size marker is lacking and probes are to long.

b) The organisation of the genetic material at the insertion site

PCR and DNA sequence analysis. The dossier refers to a report by Palmer (2003). This report describes the PCR and sequence analysis of the insert and linkage of genetic elements in Roundup Ready Canola event RT73. Here they report PCR and sequencing of the entire insert and an attempt to sequence the flanking region (5-prime). All PCR products of expected size were sent to sequencing. However, they also observed PCR products that were not expected, but these were not reported because the sequence data were un-interpretable or they only sequenced the bands of

expected size. The attempt to amplify the 5-prime flanking region produced the expected, but also un-expected PCR products. But these were not reported, only those of expected size. This tells us that they have not made an attempt to look at possible rearrangements at this site. The first 93 bp of the 5-prime region were not reported here, but in a report by Petersen in 2000. All these data are old and should be re-done.

c) All sequence info including the location of the primers used for detection.

The sequence information for the whole insert is covered by two reports by Palmer (2003) and Peterson (2000). However, sequences of the flanking regions remains a bit unclear, also they have not sequenced all PCR products outside expected size ranges. This is especially the case for the 5-prime region where there is a clearly visible band above the expected size. Analysis of potential disruption of endogenous ORFs or ORFs or regulatory regions present in the flanking regions of the oil seed rape AFTER the transformation was performed was analysed by Tu and Silvanovich (2011) using BLASTn searches with defined cut off values. They concluded that it was unlikely that ORFs or genes were disturbed by the insertion.

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

6.1. 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 GT73. 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 GT73) 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.

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 GT73. 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 GT73 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.

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/

Cummings JL, Handley LW, Macbryde B, Tupper SK, Werner SJ, Byram ZJ (2008) Dispersal of viable row-crop seeds of commercial agriculture by farmland birds: Implication for genetically modified crops. *Environ Biosafety Res.* vol 7, 4:241-52

Demeke T, Ratnayaka I (2008) Multiplex qualitative PCR assay for identification of genetically modified canola events and real-time event-specific PCR assay for quantification of the GT73 canola event. *Food Control*, vol 19: 893–897

DN report, 280508. “Rapport for slutføring av søknad C/NL/98/11 om utsetting av oljeraps (*Brassica napus* L.) linje GT73 under EU-direktiv 2001/18/EC”.

Dobert, R., Fichet, Y., Lirette, R., Doherty, S., Deng, M. (1998) Appendice CI, Study#98-01-43-03.

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 Umweltrisikoaabschätzung gentechnisch veränderter Pflanzen in der EU. BfN – pp. 259.

Hart M (2011) Farmer to Farmer. A short film on agriculture of genetically modified crops in the USA. (www.gmcropsfarmertofarmer.com)

Makarevitch, I., Svitashov, S.K. & Somers, D.A. (2003). Complete sequence analysis of transgene loci from plants transformed via microprojectile bombardment. *Plant Mol. Biol.* 52, 421-432.

Palmer, GM et al (2003) Study #:02-01-43-13 Monsanto Company.

Peterson, EA et al (2000) Study #:00-01-43-06 Monsanto Company.

SPÖK, A.; HOFER, H.; LEHNER, P.; VALENTA, R.; STIRN, S. & H. GAUGITSCH (2004). Risk assessment of GMO products in the European Union. Umweltbundesamt Wien. Berichte, Band 253. July 2004, pp 132
<http://www.umweltbundesamt.at/en/publikationen/publikationssuche/publikation>

Svitashov, S.K., Pawlowski, W.P., Makarevitch, I., Plank, D.W. & Somers, D.A. (2002). Complex transgene locus structures implicate multiple mechanisms for plant transgene rearrangement. *Plant J* 32, 433-445.

Technical Dossier; Part I, GT73.

Tu, H., Silvanovich, A. (2011) Appendice CI, RAR-2011-0192, Monsanto Company.

Twigg LE, Taylor CM, Lowe TJ, Calver MC (2008) Can Seed-eating Birds Spread Viable Canola Seed? *Pacific Conservation Biology*, Vol 14, 2:119-127.