GenØk - Centre for Biosafety

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Assessment of the technical dossier of EFSA/GMO/BE/2010/79 submitted by the applicant for MON 87701

Centre for Biosafety – GenØk
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We performed a brief technical evaluation of the nature and quality of information provided by the applicant. Current resource constraints prevented a more in depth analysis, yet we focused on a few fundamental aspects for critique.

KONKLUSJON

Genøk –Senter for Biosikkerhet viser til brev fra Direktoratet for naturforvaltning (DN) angående høring relatert til soyaplanten MON 87701 for bruksområdene import, prosessering, mat og fôr. Soyaplanten MON 87701 er en stablet GMO med en pesticid-kodende gene (Bt-toksiner) innebygd.

Informasjonen som er tilgjengelig fra søker er ikke tilstrekkelig for uavhengig evaluering av søknaden. Det foreligger ingen resultater fra analyser eller detaljerte forsøksoppsett til oppklaring av DNA sekvens, lokalisering av transgenet i soyagenomet, protein uttrykk, toksikologiske/immunologiske effekter eller foringsforsøk i relevante dyremodeller. Det er ikke opplyst hvorvidt søker har frigitt frø fra den genetisk modifiserte planten og relevante ikke-GMO kontroll planter. Dette er nødvendig for at fri og uavhengig forskning med denne planten skal være mulig.

Stablede planter har generelt en mer kompleks genetisk sammensetning og derfor større potensial for opp- og nedregulering av plantens egne gener. Derfor burde de gjennomgå grundig testing før eventuell markedsadgang. GenØk mener det ikke er faglig velbegrunnet å godkjenne stablede planten basert på at foreldrelinjene, hver for seg, er godkjent.

Det kan ikke utelukkes at gruppen av de uttrykte toksinene cry1Ac kan gi spesifikke immunogene effekter eller adjuvanseffekter (fremming av immunreaksjoner mot andre stoffer) hos pattedyr og mennesker.

Søker gir ikke opplysninger som adresserer vurderingskriteriene bærekraft, samfunnssnytte og etiske aspekter som forutsettes anvendt i den norske genteknologiloven. I denne sammenheng er det viktig å få dokumentert om den omsøkte planten fører til mindre bruk av sprøytemidler, samt erfaringer med hensyn på effekter på miljø, helse og samfunnsaspekter hos bønder som dyrker den. Denne type dokumentasjon er ikke vedlagt søknad om omsetting av MON 87701.

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Basert på vår analyse og manglende uavhengige studier og data tilgjengelig vi ønsker å påpeke at det er kunnskapshull relatert til risiko for helse og miljø ved MON 87701. Disse kunnskapshullene er spesielt relatert til usikkerhet ved effekter som kan oppstå på grunn av kombinasjonen eller synergistiske effekter av de innsatte genene og viser til at en bør bruke føre-var prinsippet og ikke godkjenne bruk i Norge.

About the plant

The parent lines contribute the *cry1Ac* from MON 87701. The inserted genes give the plant resistance to *Lepidopteran* insect herbivores.

1. The presence/absence of antibiotic resistance marker genes

The vector cassettes used in the transformation that produced event MON87701 include the antibiotic resistance (ABR) gene *aadA,* which falls into the class ABR genes designated as Group 2 by the EFSA GMO panel.

Although applicant asserts "As these elements are outside of the border regions, they are not expected to be transferred into the soybean genome." (see technical dossier, p. 29) and provide rudimentary data from Southern hybridizations, they provide no direct evidence to support the assertion. The applicant relies on the of absence of evidence as evidence of absence of the ABR gene in southern blot experiments.

Given the current stance of Norway to restrict the use of antibiotic resistance genes as selectable markers in GMOs, direct proof is necessary. The contention should be back up with reproducible, verifiable evidence. This would be easily obtained by simple PCR and sequencing with targeted primers, for example.

Recommendation 1: The applicant should provide direct proof of the absence of* aadA *antibiotic resistance marker gene.

2. Criteria for bioequivalence used in the applicants studies are inadequate for a relevant verification of environmental and non-target studies using Cry1Ac

Included in the dossier is a study of the "bioequivalence" of surrogate transgenic Cry1Ac protein derived from the bacterium E. coli, and used in many testing schemes in the place of the transgenic protein actually produced in plants (and put into the environment/consumed by other organisms).

The stated criteria for bioequivalence are stated in the applicant's study MSL0021146 by Bell et al (2008), p 21:

A. The apparent molecular weight of the full-length MON 87701-produced protein is within ± 5% of the *E. coli*-produced Cry1Ac protein.

B. The immunoreactivity of the MON 87701-produced Cry1Ac protein with Cry1Ac-specific antibodies is within ± 35% of the *E. coli*-produced Cry1Ac immunoreactivity.

C. The functional activity of the MON 87701-produced protein, expressed as an EC50 value, is

less than 3-fold different than the EC50 value for the *E. coli* produced protein.

D. The MON 87701-produced and the *E. coli*-produced Cry1Ac are not glycosylated.
If these criteria are met then the proteins are considered equivalent to one another."

Given the current state of knowledge that small changes in primary and secondary protein sequences can impart important changes in their bioactivity, and specifically changes to specificity and toxicity of the Cry1Ac protein (Haider and Ellar, 1989; Geiser et al., 1996). Therefore, the non-conservative criteria (justified by testing variablilty, yet this does not characterize well characterization techniques) are permissive of possible small but significant changes in bioactivity or immunogenicity, and represents an extremely weak standard of safety.

For example, while the conclusions of Bell et al (2008) broadly ignore small changes that warrant followup bioequivalence study, and when considered, they are not consistent with the results seen from their analysis. In the SDS-PAGE gels provided, clear differential patterns of transgenic protein degredation between the bacterial and plant versions of the Cry1Ac protein are evident (ibid, p. 43). Given that proteins, and Cry proteins in particular, undergo hydrolysis by serine proteases, the pattern (and size) of degradation would be expected to be uniform when the folding and peptides are the same for the two biological sources of Cry1Ac. This is not the case here. This suggests that any organism in contact with the Cry1Ac would be expose to degradation products that likely differ, in unknown ways and biological activities between the two Cry1Ac variants.

Lastly, the applicants means of determining glycolsylation status of the two proteins is crude at best. A more complete profile is possible using Oligosaccharide mapping, liquid chromatography, and mass spectrometry (Werner et al, 2007).

Therefore, the standard set by the applicant for bioequivalence is unacceptably low and is not justifiable, based on current scientific knowledge for the use of E. coli-produced Cry1Ac protein in studies to assess the safety Cry1Ac protein present in MON 87701 soybean.

Recommendation 2: The criteria for equivalency should be strengthened to reflect the current state of knowledge that small differences in proteins can have large biological effects. The applicant should provide more information and details as to the extent of changes evidenced in Bell et al (2008). Specifically, the applicant should perform mass spectrometry analyses of both secondary and tertiary structural information in a comparative fashion, including glycosylation analyses. This would provide a reasonable assurance of equivalency, using standard up-to-date methods for strucutural and functional determinations of proteins.

Compositional analysis
Contention of HISTORY OF SAFE USE AND CONSUMPTION OF CRY PROTEINS, p. 159

Non-target effects and effects on biodiversity from Bt-proteins

In two meta-analyses of published studies on non-target effects of Bt proteins in insects, (Lövei and Arpaia 2005) documented that 30% of studies on predators and 57% of studies on parasitoids display negative effects to Cry1Ab (another type of Cry1A protein) transgenic insecticidal proteins. A review by (Hilbeck and Schmidt

2006) on various Bt-plants found 50% of studies documenting negative effects on tested invertebrates.

Another quantitative review by (Marvier et al. 2007) suggested a reduction in non-target biodiversity in some classes of invertebrates for GM (Bt) cotton fields vs. non-pesticide controls, yet found little reductions in biodiversity in others.
Impacts on soil microflora and fauna, including earthworms (Zwahlen et al. 2003), mychorizzal fungi (Castaldini et al. 2005) and microarthropods in response to Cry endotoxins have also been reported (Griffiths et al. 2006; Wandeler et al. 2002).
The significance of tritrophic effects of accumulation, particularly of insecticidal Cry toxins (Harwood et al. 2006; Obrist et al. 2006) is, however, yet to be firmly established. It has been demonstrated that subchronic dosages of Cry proteins may affect both foraging behavior and learning ability in non-target bees (Ramirez-Romero et al. 2008), and may have indirect effects on recipient populations, and, given the key-stone role of bees as pollinators, on both primary production and on entire food-webs.

Animal and human health

A recent publication by (Dona and Arvanitoyannis 2009) reviews the potential health implications of GM foods for humans and animals, including incidences and effects of increased immunogenicity, amounts of anti-nutrients, possible pleiotropic and epigenetic effects, including possible reproductive and developmental toxicity. They conclude that while there is strong evidence for health concerns on many fronts, exposure duration have not been long enough to uncover what are likely small-effect levels. Studies should also include subjects with immunodeficiency or exposed to other stress agents.

Bt proteins and immune effects

There are no data available from the scientific literature on the genetic stability, transgene expression products or immuno-toxicological effects.

Published mouse experiments have demonstrated that Cry1Ac (member of the Cry1A class of endotoxins) can act as a powerful systemic and mucosal adjuvant useful as a carrier or adjuvant in vaccines (Moreno-Fierros et al. 2003; Vazquez et al. 1999; Vazquez-Padron et al. 1999; Vazquez-Padron et al. 2000), Rojas-Hernandez et al. 2004). In mammals, this property result in increased immunological and allergic responses. Published data also suggest that Cry proteins may inhibit development of mucosally induced suppressive immune mechanisms referred to as "oral tolerance" against innocuous food proteins (Brandtzaeg 2007).

There are a number of difficulties when it comes to studies of food allergies in humans. The frequency of food allergy in the human population is about 2 % in adults and about 5 % in children (EFSA Opinion 2004) and seems to require a

genetic predisposition. Whether the possibility/risk of food allergy increases with the presence of intestinal localized Cry proteins is not known. Therefore, one should not expect a high incidence of adverse effects in the general population due to ingestion of food containing adjuvants enhancing the development of allergy. But the use of transgenic feed maize containing multiple Cry proteins, brings up a concern whether there will be a higher incidence rate for food allergy. In addition, since Cry proteins possess adjuvant activity there may be enhanced inflammatory processes. Further, combinatorial or synergistic effects of recombinant proteins acting as adjuvants to immunostimulatory effects, or as potential allergens are areas of important coming scientific inquiry.

Immunological effects have largely focused on potential allergenicity of GMOs, rather than broader suites of immunogenic or toxicological responses. Inhalation studies, rather than oral toxicity are also largely missing from the scientific literature. One study by (Kroghsbo et al. 2008) found increased antigen-specific antibody response to Bt toxin and PHA-E lectin in a 28 and 90-day feeding study of Wistar rats.

Exposure studies conducted by the applicant

Feed study female weights

Precautionary approach to risk assessment

The Precautionary principle requires commitment to the idea that full scientific proof of a causal link between a potentially damaging operation and a long term environmental impact is not required to take action in order to avoid negative effects on health and the environment. Due to the lack of information available in the scientific literature genetic stability, expression of inserted proteins or immune effects as well as the stacked event of the *MON 87701*, we find that these uncertainties warrant further research and advice the DN to apply the Precautionary principle and deny the marketing until more scientific understanding has been published.

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. If a company would suffer additional costs, reduced incomes or delayed entrance into the marked, we claim that they are vulnerable to being biased in their hypothesis, study design, presentation and interpretation of the data.

The lack of compelling scientific information top 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 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.

Sustainability

In addition to the EU regulatory framework for GMO assessment, impact assessment in Norway follows the Norwegian gene technology act, which states that “in deciding whether or not to grant the application, significant emphasis shall also be placed on whether the deliberate release represent a benefit to the community and a contribution to sustainable development” hence it is obvious that, for the Norwegian authorities, that contribution to sustainable development should be assessed together with an evaluation of the societal utility in applications of use and release of GMOs. With the purpose to guide political decisions concerning GMO and the aim of the gene technology act, Norwegian authorities has with the basis in the biotechnology advisory boards discussion paper: “Sustainability, benefit to the community and ethics in the assessment of genetically modified organisms” (2003) elaborated in the impact assessment regulations annex 4 several questions, which should be considered in the evaluation of the application.

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, often in Third World countries. For instance, most Bt maize has been developed and tested in the US and it is difficult to translate and extrapolate risk assessment results on the toxicity of Bt maize to human and non-target organisms to other countries because there are great differences between; regional growing environments, scales of farm fields, crop management practices, local/ regional target and non-target species considered most important in the agro-ecosystem, interactions between cultivated

crops, and surrounding biodiversity. Toxicity and environmental impact data on other species (e.g. regionally appropriate non target insects, including other non-domesticated herbivores) and regional environments (local growing regions) would be needed to accurately determine toxicity and environmental impacts to local fauna of the five different Bt toxins and its degradation products, i.e. resulting from ingestion by herbivores and decomposition in the soil of plant material and root exudates. Even for target pest species from different countries or regions, sensitivities to expressed Bt toxins vary widely. Hence it cannot be expected that the same species-specific and even population-specific sensitivity to Bt toxins will apply between different environments and across continents. Local non target species like butterflies of conservation concern and heritage value may therefore be at risk.

Hence, Norwegian authorities should contact the applicant directly and require the required information in accordance with the Norwegian Gene Technology Act.

Societal utility

Soy is not very important in Norway as food, but is of high relevance to the feed and processed food industry. Although it at present is not as difficult for Norwegian importers to get soy that is free of GM, this may change in the future. The applicant of *MON 87701* argues that consumption is safe; this is supported in general by the use of other varieties containing this trait. The majority of feeding studies support the claims that the GM soy is as safe as the non-GM counterpart, although there are uncertainties as described in the beginning of this document. Another issue of importance is that the pests that the GM soy is resistant against, is not a relevant problem in Norway; hence the GM plants in question cannot solve problems in Norwegian agriculture. In other parts of the world where pests are a major problem, the use of *MON 87701* may hold promises for environmental benefits to agriculture by increasing yield. Given the use of pesticides, a reduction of inputs of pesticides is also possible, leading to reduced exposure to farmers. However, the issue is more complex due to employment of resistance management, potential resistance development among pests and with regard to the usefulness of Bt toxins against the most important pests. The cultivation of GM plants in general is also causing problems with regard to co-existence. For instance Binimelis et al. (2008) have investigated consequences on co-existence of Bt-maize in Spain among small-scale farmer and has found that co-existence is very difficult and that farmers in some areas has given up growing non-GM maize. In this context it is important to acknowledge that cultural concerns may be more significant than the functional utility, which has been highlighted with the debate concerning effects on Monarch butterflies and landrace corn in Mexico.

Conclusion

Based on the above, and with special attention to the lack of verifiable scientific proof of assertions in the application, confidence in the non-harm of this soy variety (*MON 87701*) is scientifically unjustified at this time. Further evidence of non-harm, including follow up feeding studies of longer duration and higher statistical power are needed.

Therefore, in our assessment of MON 87701 we conclude that based on the available data, including the safety data supplied by the applicant, is insufficient and equivocal in its lack of proof of toxicological affects on mammalian health, and its determinations of bioequivalency in surrogate proteins used in non-harm evaluations. We find that these effects may be biologically significant and warrant future study before claims of lack of harm can be scientifically established.

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