



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

### About the plant

The parent soy line contributes the *cry1Ac* from MON 87701. The inserted genes give the plant resistance to *Lepidopteran* insect herbivores.

### Assessment

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.

#### 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 absence of evidence as evidence of absence of the ABR gene in southern blot experiments. Integration of T-DNA backbone sequence has been reported for other cereal grains (Wu et al., 2006) and both linked a non-linked integration is possible.

Given the current stance of Norway to restrict the use of antibiotic resistance genes as selectable markers in GMOs, direct proof should be required. Reproducible, verifiable evidence 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 sequences via PCR designed to specifically detect the presence of aadA sequences.*

**2. Criteria for bioequivalence used in the applicant's studies are inadequate for verify the relevance of surrogate proteins for use in 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 *Escherichia coli*, in the place of the transgenic protein actually produced in plants (and put into the environment/consumed by other organisms).

The 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  $\pm 5\%$  of the *E. coli*-produced Cry1Ac protein.
- B. The immunoreactivity of the MON 87701-produced Cry1Ac protein with Cry1Ac-specific antibodies is within  $\pm 35\%$  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."

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 are permissive of possible small but significant changes in bioactivity or immunogenicity, and represent an extremely weak standard of safety. The applicant justifies the criteria is necessary given the variability in results obtained, as if it were commonplace in the technique used, which is not the case. The variability observed seems to come – though needs further investigation – from distinct differences in the proteins themselves.

The conclusions of Bell et al (2008) broadly ignore small changes observed in the two proteins that would warrant follow up analysis under a more rigorous standard. For example, in the SDS-PAGE gels provided, clear differential patterns of transgenic protein degradation between the bacterial and plant versions of the Cry1Ac protein are evident (ibid, p. 43). Given that Cry proteins are susceptible to hydrolysis by serine proteases, the pattern (and size) of hydrolytic products would be expected to be the same for the two biological sources of Cry1Ac. This is not the case here. As a result any organism in contact with the Cry1Ac produced in bacteria would be expose to degradation products that likely differ (and may be immunoreactive), to that of Cry1Ac produced in the host maize plant.

Lastly, the applicant's means of determining glycosylation 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 immunologic assays on differential hydrolytic products produced between the plant and bacterial versions of the Cry1Ac protein, including 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 structural and functional determinations of proteins.*

### 3. General environmental considerations

#### *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 with approx. 90% amino acid similarity and purported similarity in activity) 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), mycorrhizal 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.

These results suggest deeper investigations as to the extent of likelihood that these purported adverse effects may occur in the local context of introduction should be established.

#### **4. General animal and human health considerations**

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.

##### **4.1 Bt proteins and immune effects**

Published mouse experiments have demonstrated that Cry1Ac 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). 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 protein products, 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.

## 5. 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, direct verification of absence of ABR sequences, and non-conservative/permissive standards for surrogate protein equivalency used in testing regimes, we find that these uncertainties warrant further research application of the Precautionary principle and manage the still unknown risks through denial of the application until more scientific understanding as the the risks of these possible effects has been made available.

## 6. Sustainability aspects

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 several questions in the impact assessment as contained in (ibid, Annex 4), 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, many GM crops have 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 given differences in the 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.

## 7. Societal utility aspects

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 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 benefit of *MON87701* for Norwegian agriculture is largely irrelevant. 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 in cases in cases and years when *Lepidopteron* pest pressure is high. Given the use of pesticides, a reduction of inputs of pesticides is also possible, leading to reduced exposure to farmers and the end consumer. 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, leading to *higher* rates total pesticides being applied, rather than less (Tabashnik et al., 2009). 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.

## 8. Quality of 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 use of permissive standards in the analysis of safety and molecular characterization data is incompatible with a precautionary approach to risk assessment. A higher standard of "sound science" is warranted. A recent report by the Ad Hoc Technical expert group (AHTEG 2010) state:

"Sound science is based on transparency, verifiability, and reproducibility (e.g. reporting of methods and data in sufficient detail, so that the resulting data and information could be confirmed independently), and on the accessibility of data (e.g. the availability of relevant, required data or information or, if requested and as appropriate, of sample material)..."

The application of sound science and transparency would be of practical value, yet to this definition is not met in the submission of scientific information by the applicant.

### 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.**

**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. Please refer to the recommendations listed herein for specific suggestions on how the risk appraisal may be improved.**

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