GenØk - Senter for Biosafety

Vår ref:2010/ri/h76
Deres ref: 2010/2141 ART-BI-BRH

Assessment of the technical dossier of EFSA/GMO/UK/2009/76 submitted by the applicant for genetically modfiied soybean MON 87769, use in human food and animal feed

Centre for Biosafety – GenØk
Authors: David Quist and Jack A. Heinemann

We performed an technical evaluation on the nature and quality of information provided by the applicant. We find a number of reason to conclude the applicants appraisal of safety of MON 87769 is deficient. Our critique can be grouped in the 5 catagories below.

1. Data and/or information missing to assess the scientific validity of the applicant's conclusions of safety

Many of the results reported by the applicant are contained in unpublished studies and technical reports referenced therein. Without the full information of study design, methods and data it is difficult to evaluate the validity of the claims based on the summaries provided in the application. The full documentation should be made available.

For example:

Southern analysis of the intended transformation of the parental soy line with Plasmid PV-GMPQ1972 (starting p.46), containing the T-DNA that was used in Agrobacterium-mediated transformation to develop MON 87769, the applicant makes reference to details, including some gels, available in a molecular characterization by Girault, et al. (2009). However, this technical report was not included with the applicant information. Therefore, the information to substantiate the claims of applicant of the molecular characterization of MON 87769 are lacking, most significantly methods that detail the characterization of copy number of inserted transgenes, and absence of backbone vector sequences, such as antibiotic resistance sequence *aadA*, a promoter and coding sequence that confers resistance to spectinomycin and streptomycin.

Recommendation 1 (R1): The applicant should provide as an annex the full information of referenced technical reports used in the application necessary to independently assess and validate the analyses (also applies to discussion below).

Further, information on the genomic location of the insert in the native soybean recipient is not provided by the applicant. Although an *in silico* BLAST search was performed to locate potential open reading frames affected by the insert, a full characterization of border sequences would provide more meaningful information on potential endogenous gene disruption and anticipated unintended RNA or protein variants.

Recommendation 2 (R2):The applicant should provide a molecular characterization of border regions in order to identify the genomic region of integration into the native soy plant.

Safety and Nutrition Assessment of SDA Soybean Oil (p226) references a technical report by Monsanto (Hammond et al. 2008) but provides only summary information and not details of the study design or data. Without such information, the claim of safety of SDA soybean oil cannot be independently verified.

90-day feeding study in rats fed defatted meal from MON 87769 (p.227) references a technical study by the applicant (MSL 0021746, 2008), but provides only summary information and not details of the study design or data. Without such information, the claim of safety of MON 87769 cannot be independently verified.

42-day feeding study in broiler chickens fed defatted meal from MON 87769 (p.228) references a technical study by the applicant (MSL 21498, 2008) but provides only summary information and not details of the study design or data. Without such information, the claim of safety of MON 87769 cannot be independently verified.

Acute risk to humans from consumption of the PjΔ6D and NcΔ15D proteins (p. 185) referenced a technical report of the applicant, MSL 21314 (2008), yet details were not available to assess the given claims of no effect. The applicant should provide as an annex the full information of referenced technical reports used in the application necessary to independently assess and validate the information.

2. Assumption-based reasoning should not be substituted for scientific testing to bring greater certainty to the assessment and confidence in the results

The applicant makes repeated reference to the benefits of SDA acids and their precursors despite the lack of evidence of any benefit from the use of high SDA acid soybeans and their oil. This is well reflected in the applicants admission that:

> "The precise mechanism by which long chain omega-3 fatty acids contribute to reduced risk of CAD have not been clearly determined and it is not possible to

document the complete equivalency of EPA and DHA for all potentially relevant endpoints based upon currently available information." (p. 38)

Characterization of the PjΔ6D protein produced in MON 87769 (p. 196), the applicant reports the presence of bands in addition to the expected sized band in a Western analysis. The applicant states:

> "The band was sequenced, and the results indicated a presence of multiple sequences of similar abundance with no clear identification of any individual protein or protein fragments. While there may be degradation products of PjΔ6D within this band, it was not possible to clearly identify them." (p. 196).

Recommendation 3 (R3): Although these observed products were assumed to be spurious and insignificant events, the applicant should perform follow up identification (via MALDI- mass spectrometry analysis, e.g.) to confirm the identity and composition of the additional protein products observed.

Comparative assessment by the applicant reveals statistically significant differences in key components (starting p. 107). Several statistically significant differences were detected between soybean MON 87769 and A3525 in the compositional analysis of key components. There are too many statistically significant differences between MON 87769 and conventional soybean A3525 to assume equivalence. These differences were, as expected in several fatty acids, but also included unintended changes in a range of amino acids, nutrients, and vitamins were observed for both the 2006 and 2007 experimental test seasons reported. The applicant choose, without justification, to dismiss most of these differences by extrapolating these findings against a wider range of soy varieties, from which greater variation in composition, in order to artificially support a claim that such changes are within and range of normal variation, and hence biologically not significant:

> **"**Where statistical differences occurred, the measured analyte was compared to a confidence interval developed from the reference variety. Differences were also compared to ILSI ranges and ranges reported in literature." (p.169)

The use of broader control groups, including broad databases of reported ranges of other soy varieties for the compositional variable under study, is inappropriate for concluding MON 87769 is compositionally equivalent to the near-isogenic line A3525.

When the appropriate comparison is made, the applicant's conclusion that MON 87769 is not substantially different in composition from its near-isogenic conventional counterpart is not supported by the applicant's data. As recommended by the OECD guidance on COMPOSITIONAL CONSIDERATIONS FOR NEW VARIETIES OF SOYBEANS (OECD, 2001), substantial equivalence approaches to compositional

analysis is intended as a starting point starting point for identifying unanticipated and undesired compositional changes.

Recommendation 4 (R4): Given the evidence of such changes, the applicant should use this starting point to address the biological significance of these changes in the soy line under investigation instead of an endpoint to inappropriately conclude equivalence of MON 87769 and A3535 soy lines.

Further, the assessment did not include any follow up on the potential biological significance or reasons for these changes. The applicant should provide experimental data demonstrating that each statistically significant difference between MON 87769 and A3525 in the analysis of key components in different environments raises no safety concerns.

Lastly, in the final analysis the applicant provided comparative data from each individual site, from which meaning differences in composition can be detected, and chose to combine across geographically and environmentally heterogenous test sites, effectively burying the observed effect into a larger background of (likely environmentally-induced) variation. The applicant's inappropriate pooling of this data led them to incorrectly conclude:

> "Since the statistical differences detected in the individual-site analysis were not detected in the combined-site analysis, this suggests these differences were not indicative of a consistent plant response associated with the trait and are unlikely to be biologically meaningful in terms of increased weed potential of MON 87769 compared to the conventional soybean." (p.87)

We continue to stress that the use of genotypically uncontrolled comparisons, and comparisons made across environments, undermines the usefulness of the comparator and the scientific process.

3. From the information provided some of the applicants studies are inappropriately designed and unreliable to substantiate claims of safety

Comparisons using immune sera from subjects sensitized to conventional soy are not capable of detecting immune responses unique to MON 87769

In section 7.9.2, "Assessment of allergenicity of the whole GM plant or crop" a Quantitative ELISA Assessment of Human IgE Binding to MON 87769 Soybean, Control and Reference Soybean Extracts was performed.

In many of the comparative studies, the applicant makes use of "soybean references" and "commercial reference soybean lines" used in the various health related studies in this application may make detection of small but important effects within the

observed data difficult to detect. The applicant should provide a justification for using an extended set of unrelated controls in various comparative studies.

An allergenicity test in which the sera from “soybean allergic patients” was incubated with aqueous extracts prepared from the ground MON 87769 seed, control soybean, and “commercial reference soybean lines” and analyzed by a validated enzyme linked immunosorbent assay (ELISA) and western blot for IgE binding. Based on similarity of reaction profiles, the applicant concluded that the allergenic potential of levels of endogenous soybean allergens in MON 87769 and commercial control lines were essentially similar.

Based on our understanding of the experimental design, the study used sera from people sensitized to conventional soybean, not soybeans with elevated stearidonic acid composition. These individuals would not have mounted an immune reaction to an unknown allergen unique to high stearidonic acid soybean MON 87769. Therefore the study only provides baseline data about the generic allergenicity of soybeans, it is not capable of distinguishing the allergenic potential of MON 87769 from conventional soybean for people never exposed to MON 87769. We fail to understand the relevance of this study for demonstrating the safety of MON 87769. Moreover, the study was limited to 16 soy-sensitive individuals with unknown histories of sensitization. People could be exposed to MON 87769 in the diet and through inhalation of flour. Therefore, the study should include an assessment of the allergenic potential of MON 87769 through both dietary and inhalation sensitization.

Recommendation 5 (R5): The applicant should supply data from proper immunostimulation and allergenicity testing of MON 87769 including tests from diet and inhalation exposures.

Despite conclusions of equivalence with the conventional soybean, the applicant has not demonstrated that any other soybean in history has had this particular pattern of variance in values in this particular combination of metabolites. It is a methodological error to consider each significant difference in isolation because people do not eat the individual components of soybean; they eat the whole food or the whole oil.

Recommendation 6 (R6): We recommend that the evaluation of compositional differences be restricted to those of significance between the proper, isogenic and conventional comparator A3525 grown under identical conditions and at the same time as MON 87769, in multiple environments and over several years, and avoid diluting their significance by including inappropriate and irrelevant comparisons. The risks of the whole food rather than each significant difference in isolation should be of paramount consideration.

4. Important safety studies, or aspects of studies, concerning the likely use of products are not included by the applicant

High stearidonic acid soybeans are being proposed for use as human food and in particular for use in high temperature applications. However, in our reading we could find no safety studies by the applicant on the chemical composition of the oil after heating, feeding studies using products fried in the oil, or solid soy food products derived from MON 87769. The applicant claims that “, PjΔ6D and NcΔ15D desaturase proteins are not heat stabile and are likely to be denatured during toasting and processing” (p.185), yet, given that high stearidonic soybean oil is chemically different from oils derived from conventional soybeans, oilseed rape, sunflower, safflower and olives, it is difficult to understand how the applicant could assume safety of this food with a novel product.

Recommendation 7 (R7): The applicant should produce a safety evaluation of the chemical composition fot he oil, including target proteins, after heating, including feeding studies.

5. The estimates of possible human consumption of SDA and SDA soybean oil are calculated based on surveys of dietary habits from human populations in the United Kingdom, and are likely significantly different from the dietary habits of the Norwegian population.

Here in Norway, consumption rates of fish and fish oils, which naturally contain SDA and its precursors, may limits the necessity or desirability for foods containing enriched in omega-3 fatty acids, such as MON 87769. The value of such a product in the Norwegian context, particularly in relation to costs (regulatory and otherwise) and uncertainties, in accepting food enriched with omega-3 fatty acids in populations already consuming considerable naturally-occurring SDA in the Norwegian diet, likely does not justify introduct of MON 87769 into the Norwegian marketplace.

Recommendation 8 (R8): An estimate of current levels of omega-3 fatty acid intake among the Norwegian population would help clarify whether the applicants product would produce the intended value to the Norwegian consumer.

6. Conclusion

In conclusion, the significant informational, methodological, and analytical deficiencies of the information provided by the applicant is insufficient to substantiate reasonable claims of safety. The recommendations suggested above could vastly improve the confidence and scientific validity of the safety assessment of MON 87769 soybeans.

Finally, the value of an enhanced product of this type, where omega-3 fatty acids are already abundant in the Norwegian diet, should be strongly questioned.

References

Girault, R. L., Song, Z., Pan, A., Feng, D., Rice, J. F., Tian, Q. and Masucci, J. D. (2009) Amended report for MSL0021074: Molecular analysis of stearidonic acid producing soybean MON 87769, *Monsanto Technical Report*, **MSL0021926**, 1-64.

MSL 21314. (2008) An acute toxicity study of delta 6 desaturase and delta 15 desaturase proteins administered by the oral (gavage) route to mice, *Monsanto technical journal*, **MSL 21314**, 1-113.

MSL 21498. (2008) Comparison of broiler performance and carcass parameters when fed diets containing soybean meal produced from MON 87769, control, or reference soybeans, *Monsanto Technical Report*, **MSL 21498**, 1-124.

MSL 0021746. (2008) A 90-day feeding study in rats with Processed meal from MON 87769 soybeans, *Monsanto technical journal*, **MSL 0021746**, 1-544.

OECD, 2001. CONSENSUS DOCUMENT ON COMPOSITIONAL CONSIDERATIONS FOR NEW VARIETIES OF SOYBEAN: KEY FOOD AND FEED NUTRIENTS AND ANTINUTRIENTS. Environmental Health and Safety Publications Series on the Safety of Novel Foods and Feeds No. 2, Report ENV/JM/MONO(2001)15.