



Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2014_0325

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/NL/2011/93 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2011/93 was submitted by Monsanto on 9 February 2011 for the marketing of genetically modified soybean MON87708 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003¹. Soybean MON87708 contains a single insert expressing the dicamba mono-oxygenase proteins (DMO and DMO+27) conferring tolerance to dicamba-based herbicides.

The application was officially acknowledged by EFSA on 13 May 2011. On the same date EFSA started the formal three-month consultation period of the Member States, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). Seven experts answered positively to this request, and formulated a number of comments to the dossier, which were edited by the coordinator. See Annex I for an overview of all the comments and for the list of comments actually placed on the EFSA net on 4 August 2011.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 12 September 2013 (EFSA Journal 2013; 11(10):3355², and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 9 October 2013 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the EFSA GMO Panel, in particular in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA. The comments formulated by the experts together with the opinion of EFSA including the answers of the EFSA GMO Panel form the basis of the advice of the Biosafety Advisory Council given below.

¹ Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p.1).

² See <http://www.efsa.europa.eu/en/efsajournal/pub/3355.htm>

Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the European environment³.

2. Molecular characterisation

With regard to the molecular characterisation, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

3. Assessment of food/feed safety and nutritional value

3.1. Assessment of compositional analysis

A statistical analysis of the composition of GM soybean MON87708 showed that the amounts of some compounds for which a statistical difference was found did not fall within the equivalence limits that were set. As the differences for these compounds are small and their biochemical role is well-established, the Biosafety Advisory Council agrees with the GMO panel of EFSA that the observed differences do not raise safety concerns.

The Biosafety Advisory Council also considers that, although not required by the OECD Document on compositional considerations for new varieties of soybean (OECD, 2001), it lacks the analysis on dietary fibre. The Biosafety Advisory Council recommends the analysis on dietary fibre since this concept is widely accepted in human food studies and recommends the adaptation of the OECD consensus document accordingly.

3.2. Assessment of toxicity

The applicant provided different data to substantiate that the DMO and DMO+27 proteins are not toxic: bioinformatics studies revealed no significant similarities to known toxic proteins, DMO proteins were quickly digested by proteolytic enzymes and the results from a 28-day feeding study in which mice received a diet supplemented with a mixture of DMO and DMO+27 proteins did not reveal any signs of toxicity. The dose that was used in the 28-day feeding study represents a very substantial safety margin compared with the amounts humans are likely to consume.

Additionally, the applicant provided the results of a sub-chronic 90-day rat feeding study with whole GM soybean treated with a dicamba containing herbicide. This means that in this study both possible toxicological effects of the genetic modification (presence of the DMO proteins and possible unintended effects) and possible toxicological effects from the treatment with the accompanying herbicide (possible presence of dicamba and its metabolites) were studied. Some significant differences in clinical pathology parameters were observed between male rats fed diets containing soybean MON87708 and control animals. Without further investigation the Biosafety Advisory Council is not convinced that these findings are incidental.

Therefore, with regard to toxicity the Biosafety Advisory Council is of the opinion that the information provided is not sufficient.

³ As the application doesn't imply a cultivation of the GM crop in the EU, a full environmental assessment is not required in EFSA procedure and was not achieved.

3.3. Assessment of allergenicity

The potential allergenicity of the newly expressed DMO proteins has been assessed as well as the allergenicity of the whole GM soybean. It is unlikely that the new expressed proteins changed the allergenicity of the whole crop.

3.4. Nutritional value

Based on the provided results of a 42-day broiler chicken feeding study, the Biosafety Advisory Council is of the opinion that soybean MON 87708 does not raise nutritional concerns in the context of the intended use.

4. Monitoring

With regard to monitoring, the Biosafety Advisory Council is of the opinion that the information provided is sufficient.

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the applicant to the EFSA GMO Panel questions and considering the data presently available, the Biosafety Advisory Council is of the opinion that as a result of the absence of a sound explication of observed clinical differences between male rats sub-chronically fed with herbicide treated soybean MON87708 and the reference group, it is not possible to draw a final conclusion on the food safety of the event.

Given the scope of the application of this herbicide tolerant soybean (no cultivation in EU) and the fact that the establishment of volunteer plants would be unlikely (soybean cannot survive without human assistance and is not capable of surviving as a weed in Europe), the potential environmental release of MON87708 is unlikely to pose any threat to the European environment.

The Biosafety Advisory Council welcomes the fact that the evaluation of the safety of the herbicide and its metabolites has been performed by the EFSA Pesticides Unit under the European legislation for the marketing of pesticides (Regulation (EC) No 396/2005)⁴. The EFSA Pesticide Unit has concluded that the breakdown of dicamba based herbicides on GM dicamba tolerant soybean plants differs from the breakdown of dicamba in non-tolerant plants. It has assessed the toxicity of these breakdown products and has set a new maximum residue level (MRL) for the main metabolite of dicamba in GM dicamba tolerant soybean.

Prof. Maurice De Proft
President of the Belgian Biosafety Advisory Council

23-05-2014

Annex 1: Full comments of experts in charge of evaluating application EFSA/GMO/NL/2011/93 and comments submitted on the EFSA net (ref. BAC_2011_0719)

⁴ See <http://www.efsa.europa.eu/en/efsajournal/pub/3440.htm>

Minority declaration of P. Baret, D. Perreux and C. Vander Wauven

Considering that the consulted expert believes that there is a need for further testing in order to exclude any toxicological effect of soybean MON87708, three members of the Council consider that a negative advice should be issued.



Secretariaat
Secrétariat

N./réf. : WIV-ISP/41/BAC_2011_0719
Email : bac@wiv-isp.be

**Compilation of comments of experts in charge of evaluating
the application EFSA/GMO/NL/2011/93
and
Comments submitted on the EFSA net on mandate of the
Biosafety Council**

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 20 May 2011

Coordinator: Dr. René Custers

Experts: Eddy Decuypere (KUL), Johan Grooten (UGent), André Huyghebaert (UGent), Birgit Mertens (WIV-ISP), Peter Smet (Consultant), Wim Stevens (UIA), Frank Van Breusegem (VIB)

Domains of expertise of experts involved: Genome analysis, genetic engineering, transgene expression, human and animal nutrition, analysis of food/feed, industrial processing, toxicology, immunology, alimentary allergology, plant allergens, risk analysis, soybean

Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **EFSA/GMO/NL/2011/93** concerns an application of the company **Monsanto** for the marketing authorisation of the genetically modified **soybean MON87708** for food and feed applications under Regulation (EC) 1829/2003.

The application has been officially acknowledged by EFSA on 13 May 2011.

The scope of the application is:

- GM plants for food use
- Food containing or consisting of GM plants
- Food produced from GM plants or containing ingredients produced from GM plants
- GM plants for feed use
- Feed produced from GM plants
- Import and processing (Part C of Directive 2001/18/EC)
- Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

Depending on their expertise, the experts were asked to evaluate the genetically modified plant considered in the application on its 1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5) food and feed aspects. It was expected that the expert should evaluate if the information provided in the application is sufficient in order to state that the marketing of the genetically modified plant for its

intended uses, will not raise any problems for the environment or human or animal health. If information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

The comments are structured as in the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94).

It should be noted that all the comments received from the experts are considered in the evaluation of this dossier and in formulating the final advice of the Biosafety Advisory Council. Comments placed on the EFSA net are indicated in grey.

List of comments received from the experts

GENERAL COMMENTS

Comments/Questions of the expert(s)

Comment 1

This is a very comprehensive and thorough dossier. It is however remarkable that contrarily to the extensive detail given for several biosafety aspects, no detailed report regarding the results from the '90 day feeding study in rats' (p. 221) is available. Besides relevance to toxicology, this report clearly is of relevance also to the potential allergenic characteristics of the GM plant.

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

The demethylation of 3,6-dichloro-2-methoxy benzoic acid (or decamba herbicide) is realized by a mono-oxygenase enzyme derived from a gene from *Stenotrophomonas maltophilia* that expresses this enzyme.

The specificity of this enzyme could be questioned but this is further in the document clearly documented. Perhaps this specificity could already be mentioned under A5.

Comment 2

No questions

B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No questions

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

Is spectinomycin and streptomycin resistance ever looked for in Mon 87708 soybean? Is it enough to state that the elements causing this resistance are outside the border regions of the expression cassette of the plasmid to be sure (or not expected?) that the element aad A is not transferred into the soybean genome conferring resistance to spectinomycin and streptomycin?

Additional comment from coordinator:

There should be evidence in the dossier that the aad A element is not present in the event. This information is indeed available in chapter D.2: aadA is not present in the GM soybean.

Comment 2

No questions

D. INFORMATION RELATING TO THE GM PLANT

D.1 DESCRIPTION OF THE TRAITS AND CHARACTERISTICS WHICH HAVE BEEN INTRODUCED OR MODIFIED

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No questions

D.2. INFORMATION ON THE SEQUENCES ACTUALLY INSERTED OR DELETED

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No questions

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No questions

D.4. INFORMATION ON HOW THE GM PLANT DIFFERS FROM THE RECIPIENT PLANT IN: REPRODUCTION, DISSEMINATION, SURVIVABILITY

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No questions

D5. GENETIC STABILITY OF THE INSERT AND PHENOTYPIC STABILITY OF THE GM PLANT

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No questions

D.6. ANY CHANGE TO THE ABILITY OF THE GM PLANT TO TRANSFER GENETIC MATERIAL TO OTHER ORGANISMS

Comments/Questions of the expert(s)

Comment 1

Comparisons are made with a non-modified conventional comparator (near isogenic parental line grown under identical field conditions) and with natural ranges of commercial conventional soybean.

Comment 2

No questions

D.7. INFORMATION ON ANY TOXIC, ALLERGENIC OR OTHER HARMFUL EFFECTS ON HUMAN OR ANIMAL HEALTH ARISING FROM THE GM FOOD/FEED

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

- Small differences in the 9 amino acids observed to be different in the combined-site analysis relative to the conventional control were in line with the small decrease in total protein content.
- Observed differences in fatty acid values were small and not consistently reproduced at the individual testing sites; they are within the tolerance interval of 99% for conventional soybean varieties and therefore not considered meaningful from a food and feed safety and nutritional perspective.

- As for anti-nutrient levels, stachyose was increased and phytic acid was decreased in Mon 87708 compared to the conventional control, whether dicamba treated or dicamba-untreated Mon 87708 was compared with conventional soybean.

However, trypsin inhibitors were increased when dicamba-untreated Mon 87708 was compared with its conventional counterpart, and genistein and glycitein were increased when dicamba-treated Mon 87708 was compared with its conventional counterpart, but not the trypsin-inhibitors anymore.

Since the same control is used as comparator for both Mon 87708-treated and untreated soybean, it may be asked if this is due to “interacting” effects of the dicamba treatment with Mon 87708.

Since “dicamba-treatment” was done in addition to maintenance herbicide in the conventional counterpart as well as in the dicamba-untreated Mon 87708 (cfr. footnote 30 on p. 164 of the technical dossier), one could wonder why a direct comparison of dicamba treatment versus untreated of Mon 87708 is not done?

I understand that the focus is not on the dicamba treatment as for compositional analysis, but on the possible effect of the genetic manipulation, however a possible interacting effect of the dicamba treatment with the transgene seems to be excluded a priori in this technical dossier

Table 23: Why literature range from seed anti-nutrients especially, are so big? If for the lectine e.g. a range of 0.09 to 11.18 hemagglutinating units per mg fresh weight is given, is it then still an argument to say that the values observed fall within the literature range given? Anything will fall in such a range.

Additional comment from the coordinator:

According to EFSA guidance the following should be compared:

GM crop + dicamba ("treated")

GM crop + conventional herbicides ("untreated")

Conventional crop + conventional herbicides

But in this particular case it was done as follows:

GM crop + dicamba + conventional herbicide ("treated")

GM crop + conventional herb ("untreated")

Conventional crop + conventional herbicide

All treatments then have conventional herbicide

The addition of a regime that would not use any herbicide, I think, would not be relevant.

I presume this was done because dicamba will never be used as the only herbicide.

Comment 2

Has the amount of formaldehyde in Dicamba-sprayed beans been compared with unsprayed beans?

And what is known about the amount of formaldehyde in soybean meal (SBM)?

All values for the anti-nutrients are within the tolerance intervals of the conventional comparators.

Additional comment from the coordinator:

The enzymatic breakdown of dicamba by DMO results in the formation of formaldehyde. Even though also naturally produced in plants, formaldehyde is a toxic compound. It may therefore be relevant to measure the amount of formaldehyde in dicamba-treated and dicamba untreated soybeans, and compare this with data on naturally occurring amounts of formaldehyde in plants.

Comment 3

The genetically modified soybean Mon87708 will be further referred to as Mon soybean.

The OECD consensus document (2001) was followed for the demonstration of substantial compositional equivalence between Mon soybean and conventional crops. As mentioned before, in previous evaluations, this document covers a broad range of important components. It is however somewhat out of date as it does not take into account actual up to date knowledge in relation to the use of soybean as a human food.

The approach applied for the demonstration of compositional equivalence, is very often used in this type of dossiers. The biotechnology derived soybean is compared to a non-modified conventional comparator. Data from commercial conventional soybeans, grown under similar field conditions, as well as literature data are also considered. The study covers a comparison of seed and forage.

Samples analyzed were obtained from Mon soya, dicamba treated and untreated, and the near isogenic conventional control. In addition three conventional varieties were grown at each production site with a total of 14 varieties. Material was produced at eight sites in the US.

Compounds analyzed in beans are in line with previous dossiers and include proximates, amino acids, fatty acids, vitamin E. Analyses of anti-nutrients include raffinose, stachyose, lectin, phytic acid, trypsin inhibitors and the isoflavones daidzein, glycitein and genistein.

Remarks on the selection of constituents:

- **vitamin** analysis is limited to vitamin E, a very important vitamin in soybean oil; however taking into account future use of Mon soya as a human food such as in soydrinks, data on other vitamins, among others particular hydrosoluble vitamins, would be welcome
- no analyses are performed in the field of **minerals**.

Data on **nutrients** obtained for dicamba untreated Mon soybeans are first discussed. As no consistent differences in the level of nutrients are found, the applicant concludes that Mon soya is compositional equivalent to conventional soybean.

I agree with this conclusion for the compounds studied. I repeat my remarks about vitamins, other than vitamin E, and minerals.

A similar approach is applied for dicamba treated Mon soybeans with similar conclusions.

Anti-Nutrients have been studied in detail.

As all relevant anti-nutrients are considered, I have no comment on the selection of constituents.

I also agree with the conclusion of the applicant on the compositional equivalence of dicamba treated and untreated Mon soybeans to conventional soybeans.

Results of forage are not discussed as forage is used as animal feed, and my comments are limited to human nutrition.

Additional comment from the SBB :

Only ADF and NDF fibers have been analysed. This makes sense for feed but for food it lacks the analysis on dietary fibre.

In its previous advices the Biosafety Advisory Council has recommended the analysis on dietary fibre since this concept is widely accepted in human food studies and recommends the adaptation of the OECD consensus document accordingly.

The carbohydrates were assessed by calculation.

There are now a range of methods available for the direct assessment of carbohydrates which give more accurate information about the carbohydrate content. In its previous advices the Biosafety

Advisory Council recommended therefore the adaptation of the OECD consensus document accordingly.

The applicant only provided data for vitamin E. It is generally recognized that soybean is an important source of vitamins in the human diet, in particular vitamin E and vitamin K.

In its previous advices the Biosafety Advisory Council underlined that in the revised version of the OECD Consensus Document on Compositional Considerations for New Varieties of soybean (still under discussion at OECD level), Vitamin K is also listed as suggested constituent to be analysed related to food use. The Biosafety Advisory Council is of the opinion that data provided by the applicants should comply with the latest scientific standards.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No further comments, see D.7.1

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No questions

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

No questions

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No questions

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

The enzymatic activity of the DMO enzyme introduced being completely dependent on the correct folding of DMO and its occurrence as a trimer, it is indeed highly likely that heating/roasting during the initial processing of the soybeans will destroy all DMO enzymatic activity as claimed by the applicant (page 184: ‘... virtually all protein-containing soybean fractions are heated during processing prior to consumption by humans. Therefore, MON 87708 DMO is likely to be denatured during soybean toasting and processing ...’). Although I am quite willing to accept this claim, I have found no hard evidence in the dossier for this claim. Being of direct relevance to allergenicity but likely also to toxicology, experimental data on this issue would be welcome.

Additional comment from the coordinator

There indeed is no hard evidence that DMO is being denatured by heating processes. But it is also shown that the DMO very rapidly digests in SGF tests. So I think it would be a nice to know instead of a need to know to see whether DMO denatures in processing by heat.

Comment 3

The applicant reviews processes applied during transformation into different products. Attention is given to dehulling and flaking, oil extraction, solvent removal, production of crude oil and meal, oil refining, lecithin production, production of soy protein isolate.

This brief review covers the most significant processes. Some processes are not further discussed although mentioned in fig 20. Production of soy drinks is also missing.

The applicant states that due to the fact that there is compositional equivalence of Mon soybeans to conventional soybeans, it is highly likely that Mon soybean derived food and feed products are not different from the conventional products.

I agree with this conclusion.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

- Soybean protein isolate is used for soybean-based infant formula, a possible sensitive group .

- Desmethyltocopherols such as γ -tocopherol have been reported to possess anti-inflammatory, antineoplastic and natriuretic functions. Has the level of these tocopherols and isoflavones been compared in Mon 87708, treated and untreated with dicamba, with conventional counterpart?

- Has urease activity been compared (cfr. above)?

- Table 27: not clear in the legend of the table if the daily consumption mentioned is the daily consumption of soybean DM or of feed DM (of feed here).

- Percentage dry matter (DM) of soybean (not of feed)

- DDI is again of soybean meal.

This can be deduced from the text, but it should be better indicated in table 27 as well.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

No questions.

- Here the specificity of the Rieske-type non-heme iron oxygenase, comprised of a reductase, a ferredoxin and a terminal oxygenase is well explained.

- History of safe use, no structural similarity to known toxins, no acute toxic effects in mammals and a large margin of exposure and rapid digestion in simulated gastric fluids warrant the consideration safe for humans and animals.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

a) Degradation of the DMO protein in simulated gastric fluid (Burge et al., 2010).

The results of the study demonstrated that 98% of the DMO proteins were digested within 30 s of incubation in SGF when analyzed using a stained gel, and more than 98% was digested when analyzed using western blot with an anti-DMO antibody.

b) Degradation of the DMO protein in simulated intestinal fluid (Burge et al., 2010).

Results of this study demonstrated that more than 95% of the full-length DMO proteins were digested within 5 min of incubation in SIF.

c) DMO: Acute Oral Toxicity Study in Mice (MSL_0022527, 2010).

Conclusion:

There were no adverse effects of the DMO enzyme when administered by oral gavage at a dose of 140 mg/kg in male and female mice.

Comment 3

Protein used for safety assessment

The inserted sequence in MON 87707 encodes for dicamba mono-oxygenase (DMO), an enzyme that catalyzes the demethylation of dicamba to the non-herbicidal compound DCSA and formaldehyde. For safety testing, MON 87708 DMO purified directly from the seed of MON 87708 was used. Hence, equivalence evaluations between plant-produced and bacterial-produced MON 87708 DMO were not necessary. In mature seed extracts from MON 87708, two forms of the MON 87708 DMO protein were found. Besides the MON 87708 DMO protein with a molecular weight of 39.8 kDa, a higher molecular weight protein was present. This form consisted of the MON 87708 DMO protein plus 27 amino acids on the N-terminus originating from pea Rubisco small subunit (incorporated to improve the targeting of the precursor protein to the chloroplast) and the intervening sequences (used for cloning purposes) and was referred to as MON 87708 DMO+27 protein. For safety assessment, the term MON 87708 DMO protein referred to both MON 87708 DMO and MON 87708 DMO+27, as well as to all forms of the trimer, the active form of DMO.

Toxicological assessment of the expressed novel protein in MON 87708

Information on the primary sequence, molecular weight, substrate specificity and possible reaction products is provided by the applicant.

History of safe use of MON 87708 DMO

The newly introduced gene in MON 87708 is derived from the bacterium *Stenotrophomonas maltophilia*. The ubiquitous presence of *Stenotrophomonas maltophilia* in the environment, its occurrence in healthy individuals and its incidental presence on foods without any adverse safety reports are considered by the applicant as indications for the safety of the donor organism. In addition, the applicant states that the safety of MON 87708 DMO itself is supported by the lack of any reports of adverse effects on oxygenases, present in diets of animals and humans, that share homologies with MON 87708 DMO across all levels of protein structure.

Bioinformatic searches

Bioinformatic analyses demonstrated that MON 87708 DMO is not structurally or functionally related to toxic or allergenic proteins that adversely affect human or animal health.

Acute oral toxicity

An acute oral study was performed in CD1 mice dosed with 140 mg MON 87708 DMO protein/kg body weight. No effects related to administration of MON 87708 DMO protein were noted on survival, clinical observations, body weight, body weight gain, food consumption or gross pathology.

Based on the available data, a rodent 28 day repeat dose oral toxicity study with MON 87708 DMO was not considered necessary – no further comments.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

Since no new constituents other than MON 87708 DMO were expressed in Soybean MON 87708, a toxicological assessment is not applicable.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

No questions

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

a) 42-Day feeding study in broiler chickens (MSL0022551, 2010).

Although statistical significant differences are observed, these seem to be unrelated to the genetic modification.

b) 90-Day rat feeding study (MSL0022868, 2010).

Mortality

All animals survived to the scheduled necropsy.

Clinical Observations

None related to the test substance.

Body Weights

No differences in body weight data, which could have been assessed as being related to treatment and of toxicological concern, were measured.

Food And Test Substance Consumption

Although sporadic differences were noted, no dose-response relationship could be established.

Clinical Pathology

Hematology And Coagulation

Monocytes in female animals in the 15% group seem to be rather low, whereas eosinophil count in male test subjects in both the 15% and 30% group is increased (only statistically significant for the 30% feeding group).

The monocyte count seems to be of no concern as no dose-response relationship is observed.

The number of eosinophils is elevated only in male rats, both compared to the control and the references. What about the amount of formaldehyde in the soybean meal? Can this exert an effect on granulocytes? Why only in male subjects? This has to be further investigated.

Serum Chemistry

The alanine aminotransferase (ALT) activities are higher compared to the control as well as the references and statistically significant for the 30% feeding group. As for the eosinophil count, only male rats are affected. Further investigation is needed.

Urinalysis

No dose-response relationship could be established.

Anatomic Pathology

Macroscopic Examination

There were no test substance-related macroscopic findings.

Organ Weights

The mean absolute and relative spleen weights in the 15% test group females were lower than those observed in the 15% control group females. There was an absence of a dose-response relationship.

Microscopic Examination

All findings noted were either single observations or they were biologically equally distributed between control and treatment groups.

Comment 3

90-day feeding study in rats

A 90-day rodent feeding study was performed following Good Laboratory Practices regulations. The aim of the study was to establish if processed soybean meal from MON 87708 is as safe as processed meal from a near-isogenic conventional soybean variety A3525. Eight groups of Sprague-Dawley rats (12 ♂ and 12 ♀) were used: two test groups receiving diets containing processed meal from MON 87708 on a weight/weight basis at \pm 15% or 30%, two concurrent control groups offered processed meal from A3525 formulated into the diet on a weight/weight basis at \pm 15% or 30% and two reference groups receiving processed meal from the conventional soybean varieties Williams 82 or Hoegemeyer 274 formulated into the diet on a weight/weight basis at \pm 15% or 30%. There were no test substance-related deaths or effects on clinical observations, body weights, food consumption, clinical pathology parameters, organ weights, macroscopic findings or histopathological findings.

No comments on the 90-day study.

42-day feeding study using boiler chickens

Eight treatment groups of 100 birds received diets containing soybean meal either produced from MON 87708, from the near isogenic conventional control soybean A3525 or from 1 of the 6 commercial conventional soybean varieties. Results from the study showed that soybean meal produced from MON 87708 was nutritionally similar to soybean meal prepared from conventional soybean.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

The applicant did a thorough job in assessing the risk for allergenicity. Bioinformatics searches for putative fusion proteins and polypeptides resulting from regions flanking the insert were performed in allergen databases. Also the DMO protein itself was searched for the presence of sequence and structure similarity with known allergens. The risk for increased allergenicity of the whole plant was verified by quantifying in the GM plant the levels of allergens endogenous to conventional soybean by ELISA and 2D Western blot on the basis of immunoreactivity of serum IgE from soybean allergic individuals. Finally, being intended for human food, the digestibility of purified DMO protein in simulated gastric and intestinal fluid was verified. All these analyses did not reveal an increased risk for allergenicity deriving from the transprotein or from the whole GM plant.

Yet, the applicant insufficiently addressed the likelihood that due to DMO enzymatic activity, new (protein) derivatives are generated in the GM plant with potential allergenicity. The applicant effectively tested several potential non-protein endogenous substrates (section 7.8.1.iii) but omitted potential protein substrates from this analysis. With regard to allergenicity, a convincing experimental approach to this end would consist of immunization of rats with MON 87708 water soluble extract, followed by comparative 2D Western blot analysis of GM and parental plant extracts using the rat antiserum for immunodetection. Such approach would provide more firm evidence for the absence or not of novel protein derivatives generated in the GM plant as a result of DMO enzymatic activity.

Comment 3

Soy is very well known as an allergenic plant especially in children. There are different soy allergen with possible cross reactivity. This is acknowledged by Monsanto and the allergenicity to the modified plant was demonstrated with sera of known allergic patients. However, little data are given on these sera (total serum IgE, level of soya allergen specific IgE – to which allergen? – mono- or polysensitised patients? Age of the patients? Disappearance of binding after heating sera?

In addition, recently a basophil activation technique has been described which is much more sensitive than the techniques applied hitherto (Sabato et al., 2011). It might be worthwhile to apply this technique to the modified protein in order to be able to pick up very low amounts of cross reacting IgE.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

No questions.

The nutritional equivalence of soybean meal produced from Mon 87708 and from conventional soybean is convincingly proven.

D.7.11 Post-market monitoring of GM food/feed

Comments/Questions of the expert(s)

Comment 1

No questions

D.8. MECHANISM OF INTERACTION BETWEEN THE GM PLANT AND TARGET ORGANISMS (IF APPLICABLE)

Comments/Questions of the expert(s)

Comment 1

Not applicable

D.9. POTENTIAL CHANGES IN THE INTERACTIONS BETWEEN THE GM PLANT WITH THE BIOTIC ENVIRONMENT RESULTING FROM THE GENETIC MODIFICATION

D.9.1. Persistence and invasiveness

Comments/Questions of the expert(s)

Comment 1

No questions

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

No questions

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

No questions

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

No questions

D.9.5 Interactions of the GM plant with non-target organism

Comments/Questions of the expert(s)

Comment 1

No questions

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

No questions

.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

No questions

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

No questions

D.9.9 Impacts of the specific cultivation, management and harvesting techniques

Comments/Questions of the expert(s)

Comment 1

No questions

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

Not applicable

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

No questions

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

No questions

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

No questions

D.11.4 General surveillance of the impact of the GM plant

Comments/Questions of the expert(s)

Comment 1

No questions

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

No questions

References

Sabato V, van Hengel AJ, De Knop KJ, Verweij MM, Hagendorens MM, Bridts CH, De Clerck LS, Schiavino D, Stevens WJ, Ebo DG (2011) Human basophils: a unique biological instrument to detect the allergenicity of food. *J Investig Allergol Clin Immunol*, 21(3):179-84.