Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2015_0811

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/NL/2012/108 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2012/108 was submitted by Monsanto on 29 March 2012 for the marketing of genetically modified (GM) soybean MON87708 x MON89788 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003[1].

Soybean MON87708 x MON89788 is a stacked event obtained by conventional crossing (no new genetic modification involved) of two single soybean events MON87708 and MON89788. It expresses the DMO protein conferring tolerance to dicamba-based herbicides and the CP4 EPSPS protein conferring tolerance to the herbicidal active substance glyphosate.

The application was officially acknowledged by EFSA on 20 July 2012. On 23 September 2013 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).

The Belgian Biosafety Advisory Council (BAC) did not participate in this consultation.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 27 May 2015 (EFSA Journal 2015; 13(6):4136[2]), and published together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.

In the frame of the preparation of this advice, the BAC, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts chosen from the common list drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). The experts were invited to evaluate the dossier, taking also into account the EFSA opinion and the two advices already published by the BAC on the single events MON87708[3] and MON89788[4]. Three 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 the comments.

[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/4136.htm
[3] Advice of the Belgian Biosafety Advisory Council of 21 May 2014 on the application EFSA/GMO/NL/2011/93 from Monsanto under Regulation (EC) No. 1829/2003 (ref WIV-ISP/BAC/2014_0325)
[4] Advice of the Belgian Biosafety Advisory Council of 26 September 2008 on the application EFSA/GMO/NL/2006/36 from Monsanto under Regulation (EC) No. 1829/2003 (ref WIV-ISP/BAC/2008_813)

The advice of the Biosafety Advisory Council given below is based on:

- The comments formulated by the experts
- The opinion of EFSA
- The two advices already adopted by the BAC on the single events MON87708 and MON89788. The conclusions of the BAC were as follows:
  - For soybean MON87708, the BAC concluded 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 was not possible to draw a final conclusion on the food safety of the event.
  - For soybean MON89788, the BAC agreed with the conclusion of the GMO panel of EFSA that it is unlikely that soybean MON89788 will have any adverse effect on human and animal health or on the environment in the context of its proposed uses, provided that the feeding trials have been conducted with GM soybean treated with glyphosate and non-GM soybean treated with conventional herbicides.

MON87708 and MON89788 are both authorised in the EU for food and feed uses with the exception of GMO cultivation[5].

Scientific evaluation

1. Environmental risk assessment

The Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of soybean MON87708 x MON89788 seeds (i.e. during transport and/or processing) into the European environment[6] will lead to any unwanted effects.

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

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM soybean MON87708 x MON89788, in comparison with its conventional counterpart, 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, 2012), 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

In its advice on the single event MON87708, the Biosafety Advisory Council expressed some concerns regarding the results of the sub-chronic 90-day rat feeding study with the whole

[5] EU register of GM food and feed: http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=63 for MON87708 and http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=32 for MON89788

[6] As the application doesn't imply a cultivation of the GM crop in the EU, a full environmental assessment is not required according to EFSA procedure and was therefore not achieved.

beans derived from the GM soybean. Some significant differences in clinical pathology parameters were observed between male rats fed diets containing soybean MON87708 and control animals. The BAC concluded that without further investigation it was not convinced that these differences were incidental.
Since no new information has been provided in the current application in relation with the toxicological assessment of the whole food derived from GM soybean MON87708 x MON89788, the concerns expressed above are still valid.
As a consequence, the Biosafety Advisory Council is unable to determine whether GM soybean MON87708 x MON89788 is as safe as conventional soybean from a toxicological perspective.

3.3. Assessment of allergenicity

The Biosafety Advisory Council agrees with the EFSA GMO Panel that there are no indications that GM soybean MON87708 x MON89788 would have an allergenic profile that would be significantly altered in comparison with its conventional counterpart.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that there are no indications that the GM soybean MON87708 x MON89788 would be less nutritious than conventional soybean varieties.

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 two advices already adopted by the BAC on the single events MON87708 and MON89788, and considering the data presently available, the Biosafety Advisory Council is of the opinion that as a result of remaining uncertainties concerning the toxicity of the whole food derived from the GM plant, it is not possible to draw a final conclusion on the food safety of soybean MON87708 x MON89788.

Given the scope of the application of this GM 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 soybean MON87708 x MON89788 is unlikely to pose any threat to the European environment.

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

Annex I: Minority declaration
Annex II: Comments of experts in charge of evaluating application EFSA/GMO/NL/2012/108 (ref. BAC_2015_0812)

Minority declaration of P. Baret and D. Perreaux

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

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Email. : bac@wiv-isp.be

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2012/108

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 8 September 2015.
Coordinator: René Custers
Experts: Eddy Decuypere (KUL), André Huyghebaert (UGent), Peter Smet (Consultant).
Domains of expertise of experts involved: Animal and human nutrition, food/feed processing, toxicology, general biochemistry, statistics.
SBB: Didier Breyer, Fanny Coppens, Martine Goossens, Katia Pauwels.

♦ INTRODUCTION

Dossier **EFSA/GMO/NL/2012/108** concerns an application submitted by the company **Monsanto** for authorisation to place on the market genetically modified **soybean MON87708 x MON89788** in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed.

EFSA declared the application valid on 20 July 2012 and published its final opinion on this application on 18 June 2015 (EFSA Journal 2015; 13(6):4136).

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) allergenicity, 3) toxicity and/or 4) 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). Items are left blank when no comments have been received either because the expert(s) focused on other related aspects, or because for this dossier the panel of experts who accepted to evaluate the dossier didn't have the needed expertise to review this part of the dossier.
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.

The attention of the experts was drawn on the fact that this application concerns a GM plant containing a combination of two transformation events ("stacked transformation event"). The data for the single events have already been assessed by the Biosafety Council, resulting in the following deliverables:
- Soybean MON 87708 (EFSA/GMO/NL/2011/93): Council's advice published on 21/05/2014. This GMO is authorised for commercialisation in the EU since 24/04/2015;
- Soybean MON 89788 (EFSA/GMO/NL/2006/36): Council's advice published on 26/09/2008. This GMO is authorised for commercialisation in the EU since 04/12/2008.
They were informed in particular that the Biosafety Council was unable to reach a final conclusion on the food safety of the single event MON 87708 due to uncertainties in the results of the sub-chronic 90-day rat feeding study.

Since comments from experts were requested after the publication of the EFSA's opinion, they were not sent to EFSA and used directly by the Biosafety Council as a scientific basis to draft its final advice on this application.

List of comments/questions received from the experts

GENERAL COMMENTS

No comments.

A. HAZARD IDENTIFICATION AND CHARACTERISATION

A.1. INFORMATION RELATED TO THE RECIPIENT OR (WHERE APPROPRIATE) THE PARENTAL PLANT

Comment
No questions.

A.2. MOLECULAR CHARACTERISATION

A.2.1. INFORMATION RELATING TO THE GENETIC MODIFICATION including:

- Description of the methods used for the genetic modification
- Source and characterization of nucleic acid used for transformation
- Nature and source of vector(s) used

Comment
No questions.

A.2.2. INFORMATION RELATING TO THE GM PLANT including:

- Description of the trait(s) and characteristics which have been introduced or modified
- Information on the sequences actually inserted or deleted
- Information on the expression of the insert
- Genetic stability of the inserted/modified sequence and phenotypic stability of the GM plant

Comment
MON87708 x MON89788 is produced by crossing both parental lines using traditional breeding; It combines tolerance to the dicamba trait (herbicide) and glyphosate-tolerance trait, by expressing DMO x CP4EPSPS.
DMO is a non-heme iron oxygenase comprised of a reductase, a ferredoxin and a terminal oxygenase, performing demethylation of an electron acceptor substrate, dicamba; therefore it is a redox system, producing the non toxic formaldehyde and 3,6 dichloorsalicylic acid (DCSA). MON87708 expresses a functional DMO that confers dicamba tolerance
CP4EPSPS: results in a much reduced affinity to glyphosate compared to endogenous plant EPSPS, essential for the biosynthesis of aromatic amino acids (phenylalanine, tryptophane, tyrosine); CP4EPSPS is targeted to the chloroplast were EPSPS resides, via a CTP2-CP4-EPSPS precursor protein

A.3. COMPARATIVE ASSESSMENT

A.3.1. CRITERIA FOR THE SELECTION OF COMPARATOR(S)

Comment 1
A3525, the near isogenic line to MON88708 x MON89788 was used as conventional counterpart, + conventional reference varieties.

Comment 2
Soybean MON87708 x MON89788 will be further referred as soybean 108.
Soybean 108 was obtained by traditional breeding of two parental lines MON87708 and MON89788. As both lines have been evaluated preciously in terms of compositional and nutritional equivalence, it is rather unlikely that differences will be found.
Soybean 108 was compared to a conventional soybean counterpart with similar background genetics and with other conventional soybeans.

A.3.2. FIELD TRIALS: EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Comment 1
OK; MON87708 x MON89788 was either untreated or treated with both dicamba and glyphosate.

Comment 2
No remarks.

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1
- Table 9: Why is the MON89788 and near isogenic control so high for values of all fatty acids, but not for amino acids for USA 2005? Also the values for fibre of seeds is higher and also lectin?
This is not addressed in the text on p. 50, 51, 52.
- For the remaining data: confirming compositional similarity of MON87708 x MON89788 with control or reference varieties; if there are any differences, then in the context of variability in the range of individual replicate values for the control, they have no relevance from a food or feed perspective (e.g. for daidzein).
- On page 52, first line, the text refers to table 5, but is it not table 7?
- On page 58, again on line 14, the text refers to table 6 now, but is it not table 7?

Comment 2
The OECD guidelines are followed for the selection of compounds and characteristics. No unexpected changes have been observed.
Relevant nutrients, proximate, amino acids, fatty acids, anti-nutrients are included. Vitamin analysis is limited to vitamin E.
Results of analysis of soybean 108 treated with dicamba and glyphosate and soybean 108 not treated with cicamba and glyphosate are discussed in detail.
It is concluded that soybean 108 is compositionaly equivalent to conventional soybean.
I agree with this conclusion.

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comment
No questions.

A.3.5. EFFECTS OF PROCESSING

Comment 1
No questions.

Comment 2
As no differences have been found between soybean 108 and conventional soybeans, the processing of soybean 108 is not expected to be different from conventional soybean.
No further remarks.

A.4. TOXICOLOGICAL ASSESSMENT

A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

Comment
No questions.

A.4.2. ASSESSMENT OF NEWLY EXPRESSED PROTEINS including:

- Molecular and biochemical characterisation of the newly expressed proteins
- Up-to-date bioinformatic search for homology
- Information on the stability of the protein under the relevant processing and storage conditions for the food and feed derived from the GM plant
- Data concerning the resistance of the newly expressed protein to proteolytic enzymes
- Repeated dose toxicity studies using laboratory animals

Comment
- Why saying (on page 85) that CP4EPSPS is less sensitive to inhibition by glyphosate compared to plant endogenous EPSPS in view of no differences at all in aromatic amino acids between treated or untreated MON87708 x MON89788 or MON89788 or untreated isogenic controls?
Why not completely unsensitive? (instead of LESS sensitive)?
- Repeated dose toxicity in lab animals:
    - NOAEL
    - History of safe consumption
    - Not structurally or functionally realted to toxic or allergenic proteins
    - Complete digestion in simulated gastric and intestinal fluid
    - No interaction between these 2 proteins known or conceivable mechanisms of interaction
    - No toxicity in acute mouse toxicity study at high doses

Therefore no need for another 28-day repeat dose oral toxicity study with MON87708 x MON89788.

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

Comment
Not applicable.

A.4.4. ASSESSMENT OF ALTERED LEVELS OF FOOD AND FEED CONSTITUENTS

Comment
Not applicable.

A.4.5. ASSESSMENT OF THE WHOLE FOOD AND/OR FEED DERIVED FROM GM PLANTS

Comment 1
No questions; I agree that a new 90-day feeding study in rodents with MON87708 x MON89788 is not warranted; it would be a waste of time, efforts and money, certainly also in view of the aim of reducing the number of laboratory animals used.

Comment 2
Additional note SBB: The comment below refers to the 90-day feeding study performed with the single event MON87708 (in the frame of application EFSA/GMO/NL/2011/93). Since the Biosafety Council was unable to conclude in 2014 on the food safety of MON87708 due to uncertainties in the results of this feeding study, the experts were asked to evaluate whether these uncertainties were still relevant and could raised safety concerns in the context of the evaluation of GM Soybean MON87708 x MON89788.
No additional 90-day feeding study in rodents was performed with MON87708 × MON89788.

- To my point of view, no new information was provided concerning the 90-day rat feeding study.
- The effects seen in the 90-day rat study are probably not induced by the DMO enzyme, because no toxic effects were seen during the acute test.
- Referring to the 90-day rat study, the following effects should be highlighted:
    - There was an increase in eosinophil count
    - There was an increase in serum ALT level
    - A dose-respons relationship was observed
    - Only male rats were affected
- Formaldehyde is a metabolite from the DMO breakdown of dicamba.
- It appeared to be very difficult to find toxicity data in literature for formaldehyde under similar conditions as those used during the 90-day study (dossier 93). Nevertheless, some results are worth mentionning:
    - Male rats are often easier affected than female species (http://www.atsdr.cdc.gov/toxprofiles/tp111.pdf consulted on 28/09/2015)
    - Exposure to formaldehyde has shown to increase the number of eosinophils (Environmental Toxicology and Pharmacology, Volume 24, Issue 2, September 2007, Pages 174–182).
    - Exposure to formaldehyde has shown to increase the serum alanine-amino transferase (ALT) level (Environment International 35 (2009) 1210–1224).
- None of these references provide hard evidence that formaldehyde is at the basis of the effects seen in dossier 93. Nevertheless, it seems to be suspicious.
- Conclusion: further investigation is needed. In a first step the amount of formaldehyde in dicamba-sprayed feed should be determined.

Additional note SBB: Following further discussion on these issues between the Council's members, the expert was asked to clarify (i) the possible safety concerns associated with the findings in the subchronic feeding study with the single event MON87708 and (ii) the possible role of formaldehyde. The answer was as follows:

Indeed, formaldehyde is volatile but I assume that dicamba is taken up by the plant cells where it is converted by the DMO system to formaldehyde which easily dissolves in the cytoplasma of the cell. I have no idea of the accumulation potential of this molecule.

When looking at the data of the 90-day rat study, ignoring the possible presence of formaldehyde, the following effects can be seen:
1) A raised alanine aminotrasferase (ALT) activity in both the 15% and 30% test group. Since the difference in the 15% test group (Mean 43 U/L ALT) is not significant compared to the 15% control group (Mean 41 U/L ALT) this group cannot be used to draw a conclusion. On the other hand, the 30% test group (Mean 41 U/L ALT) shows a statistical significant increase compared to the 30% control group (Mean 49 U/L ALT). The mean value of the 30% test group falls outside the 30% historical control data (Mean min/max 38/47 U/L ALT; Population grand mean 44 U/L ALT).
   a) Since ALT is a specific marker for hepatic parenchymal injury induced by xenobiotics, something must have triggered this effect in the male species of the rat study. There has been a negative effect on their liver which cannot be simply ignored by using the argument that it is absent in female species.
   b) Although the effect in the 15% test group is not significant, the mutual results of both groups (15% and 30%) suggest a dose-respons relationship.

I would be very carefully to interpret these findings as coincidental.
2) The number of eosinophils is elevated only in male rats, both compared to the control and the references. However, the mean of the 30% test group falls within the historical control data.

I) To my point of view the company should provide scientific data indicating that these effects are incidental (or not).
II) Can't they determine the amount of formaldehyde (and its metabolites) in sprayed and non-sprayed soybean and its derived products (feed)?

A.5. ALLERGENICITY ASSESSMENT

A.5.1. ASSESSMENT OF ALLERGENICITY OF THE NEWLY EXPRESSED PROTEIN including:

- Amino acid sequence homology comparison between the newly expressed protein and known allergens using a comprehensive database
- Specific serum screening
- Pepsin resistance and in vitro digestibility tests
- Additional tests

Comment
No questions.

A.5.2. ASSESSMENT OF ALLERGENICITY OF THE WHOLE GM PLANT

Comment
No questions.

A.5.3. ADJUVANTICITY

Comment
No questions.

A.6. NUTRITIONAL ASSESSMENT

A.6.1. NUTRITIONAL ASSESSMENT OF FOOD DERIVED FROM GM PLANTS

Comment
No questions; I agree with the conclusion in 6.3.

A.6.2. NUTRITIONAL ASSESSMENT OF FEED DERIVED FROM GM PLANTS

Comment
No questions.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comment
No questions.

C. RISK CHARACTERISATION

Comment
No questions.

D. POST MARKET MONITORING (PMM) OF FOOD AND FEED DERIVED FROM GM PLANTS

Comment
No questions.

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comment
No questions.

E.2. GENERAL APPROACH OF THE ERA

Comment
No questions; see remarks earlier under A.3.3.

E.3. SPECIFIC AREAS OF RISK

As stated in the EFSA guidance on the environmental risk assessment of genetically modified plants (EFSA Journal 2010, 8(11):1879) the objective of the ERA is on a case-by-case basis to identify and evaluate potential adverse effects of the GM plant, direct and indirect, immediate or delayed (including cumulative long-term effects) on the receiving environment(s) where the GM plant will be released. For each specific risk the ERA consists of the six steps described in Directive 2001/18/EC:
1. Problem formulation including hazard identification,
2. Hazard characterisation,
3. Exposure characterisation,
4. Risk characterisation,
5. Risk management strategies,
6. Overall risk evaluation and conclusions.

E.3.1. PERSISTENCE AND INVASIVENESS INCLUDING PLANT-TO-PLANT GENE FLOW

Comment
No questions.

E.3.2. PLANT TO MICRO-ORGANISMS GENE TRANSFER

Comment
No questions; table 22 is very helpful as summarizing instrument (see p. 132).

E.3.3. INTERACTION BETWEEN THE GM PLANT AND TARGET ORGANISMS

Comment
Not applicable.

E.3.4. INTERACTION BETWEEN THE GM PLANT AND NON-TARGET ORGANISMS (NTOS)

Comment
No questions.

E.3.5. IMPACTS OF SPECIFIC CULTIVATION AND MANAGEMENT AND HARVESTING TECHNIQUES

Comment
Not applicable.

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comment
Not applicable.

E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

Comment
No questions.

E.3.8. Overall risk evaluation and conclusions

Comment
No questions.

E.4. Post Market Environmental Monitoring plan

E.4.1. Interplay between environmental risk assessment and monitoring

Comment
No questions.

E.4.2. Case-specific GM plant monitoring

Comment
No questions.

E.4.3. General surveillance for unanticipated adverse effects

Comment
No questions.

E.4.4. Reporting the results of monitoring

Comment
No questions.