Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-DE-2011-95 from Syngenta Crop Protection AG under Regulation (EC) No. 1829/2003

Adopted on 29 May 2018
Ref. SC/1510/BAC/2018_0327

Context

Application EFSA-GMO-DE-2011-95 was submitted by Syngenta on 7 April 2011 for the marketing of genetically modified (GM) maize 5307 for food and feed uses, import and processing (excluding cultivation) within the European Union (EU), within the framework of Regulation (EC) No. 1829/2003[1].

Maize 5307 expresses eCry3.1Ab, a chimeric protein based on a modified Cry3A and the Cry1Ab from *Bacillus thuringiensis*, conferring resistance to certain coleopteran insect pests, and the PMI protein, used as a selection marker.

The application was officially acknowledged by EFSA on 21 June 2011 and a formal three-month consultation period of the Member States was started, lasting until 21 September 2011, 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 Service Biosafety and Biotechnology (SBB). Seven experts answered positively to this request, and formulated a number of comments to the dossier. See Annex I for an overview of all the comments, and those that were sent to EFSA.

The initial opinion of the EFSA Scientific Panel on GMOs was adopted on 16 April 2015 (EFSA Journal 2015;13(5):4083[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 its opinion, the EFSA GMO Panel could not conclude on the safety of the eCry3.1Ab protein due to weaknesses in the provided 28-day rat oral toxicity study, but indicated that maize 5307 is unlikely to have an adverse effect on the environment in the context of this application.

The applicant provided a supplementary 28-day toxicity study in mice on the eCry3.1Ab protein to the European Commission on 8 December 2016, and the Commission mandated EFSA to assess this new study on 23 December 2016 in order to complement its scientific opinion adopted on 16 April 2015. Following this, EFSA adopted on 7 March 2018 a complementing statement to its scientific opinion, which was published on 11 April 2018

[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 https://www.efsa.europa.eu/en/efsajournal/pub/4083

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(EFSA Journal 2018; 16(4):5233[3]). EFSA's complementing statement and the applicant's 28-day mouse feeding study were assessed by two toxicology experts from the common list of experts.

The comments formulated by the experts during the initial 3-month member-state consultation period and following the publication of EFSA's complementing statement, together with the overall opinion of EFSA, as well as the advices already adopted by the BAC on events expressing the PMI protein, form the basis of the advice of the Biosafety Advisory Council given below.

Scientific evaluation

1. Environmental risk assessment

The Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of maize 5307 (i.e. during transport and/or processing) into the European environment[4] 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 maize 5307, in comparison with its conventional counterpart, does not raise safety concerns.

3.2. Assessment of toxicity

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the available data on the toxicity of GM maize 5307, in comparison with its conventional counterpart, does not raise safety concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the available data on the allergenicity of GM maize 5307, in comparison with its conventional counterpart, does not raise safety concerns.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that the information provided is sufficient to conclude that the nutritional characteristics of maize 5307-derived food and feed are not expected to differ from those of conventional maize varieties.

[3] See https://www.efsa.europa.eu/en/efsajournal/pub/5233
[4] 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 performed.

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 and complementing statement of EFSA and the advices already adopted by the BAC on other GM events expressing the PMI protein, and considering the new information provided by the applicant, the Biosafety Advisory Council is of the opinion that in the context of its proposed uses, maize 5307 is unlikely to pose any risk to human and animal health.

The Biosafety Advisory Council did not identify any risk that the import and processing of this GM maize could pose to the European environment.

In addition the Biosafety Advisory Council recommends following up any unanticipated allergenicity aspects of the GM maize in the existing allergenicity monitoring systems.

Prof. Corinne Vander Wauven
President of the Belgian Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of evaluating the application EFSA/GMO/DE/2011/95 and Comments submitted on the EFSAnet on mandate of the Biosafety Council (ref. BAC_2011_0870)

Bioveiligheidsraad
Conseil de Biosécurité

19 September 2011

Secretariaat
Secrétariat

N./réf. : WIV-ISP/41/BAC_2011_0870
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/DE/2011/95
and
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 30 June 2011
Coordinator: Prof. dr. ir. Dirk Reheul
Experts: Armand Christophe (UGent), Jacques Dommes (ULg), Leo Fiems (ILVO), Rony Geers (KUL), Peter Smet (Consultant), Jan Van Doorsselaere (KATHO), Michel Van Koninckxloo (HEP Hainaut-Condorcet)
Domains of expertise of experts involved: Breeding techniques, molecular characterisation, plant biology, human nutrition, animal nutrition, toxicology in vitro, general biochemistry, agronomy, agro-ecology, maize
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **EFSA/GMO/DE/2011/95** concerns an application of the company **Syngenta** for the marketing authorisation of the genetically modified **maize 5307** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 21 June 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 EFSAnet are indicated in grey.

List of comments received from the experts

GENERAL COMMENTS

Comments/Questions of the expert(s)

Comment 1

Question not limited to this dossier.
In case it is known that a food is prone to become infested by an organism that causes toxicity, would there not be a requirement to evaluate whether a GMO is not more prone to infestation than its non-GM counterpart? (For this dossier, see D 7.3). Large differences in mycotoxin levels have been found in GMOs and their non-GMO comparators (Folcher et al., 2010).

Additional comment from the SBB:
The EFSA guidelines on Risk assessment of GM food/feed states: « The comparative assessment of the GM plant, containing single or stacked events, with its comparator should address also aspects of the biology of the plant, in the form of agronomic and phenotypic traits (e.g. yield, plant morphology, flowering time, day degrees to maturity, duration of pollen viability, response to plant pathogens and insect pests, sensitivity to abiotic stress). The protocols for field trials to study these characteristics should follow the specifications made under Section 3.1.3.2. Additional information on agronomic traits of the GM plant should be provided from additional field trials, where appropriate. »

The publication of Folcher concerns a comparison between Bt maize MON 810 and its isogenic non-Bt counterpart. Much less mycotoxins are detected on Bt maize. This is not a surprise because toxigenic fungi (Fusarium…) develop on damaged plant tissue and insects play a role in the dispersion of the fungi. The same results can probably be expected with maize 5307 modified to resist to coleopteran pest.
This point has however not been addressed in the application but it is also not required because it is not a risk but rather a benefit.

Comment 2

The chance that the use event 5307 maize for feed and food purposes will be detrimental for animal and human health, may be limited based on a series of studies, including:
- Characterization of the mode of action of eCry3.1Ab and PMI
- Acute oral mouse toxicity studies
- Molecular and biochemical characterization
- Searches for homology to known toxins and allergens that affect human or animal health
- Stability during processing
- Resistance to proteolytic degradation

eCry3.1Ab and PMI proteins are present in low levels in food and feed so that exposure to these proteins would be minimal.

Furthermore, eCry3.1Ab protein is a fusion between a modified Cry3A protein (mCry3A), and Cry1Ab protein. The safety of the mCry3A and Cry1Ab proteins has already been assessed by EFSA (EFSA, 2009a; EFSA, 2009b).

However, a limited number of in vivo tests have been conducted to evaluate event 5307 maize:
- Only 10 mice were used to test the acute toxicity of eCry3.1Ab
- Only 10 mice were used to test the acute toxicity of PMI
- Only 180 broilers were fed a diet containing event 5307 maize
- Only these 2 tests with living animals were mentioned in this dossier

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No comment

Comment 3

The name of the responsible person for all dealings with EFSA is lacking (in the cover letter?)

Additional comment from the SBB
The name of the responsible person is mentioned in the cover letter.

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

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

The information provided in the application is sufficient.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

No comment

Comment 2

No comments

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

Clear description. No questions.

Comment 2

No comment

Comment 3

No comments

Comment 4

The information provided in the application is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

Copy number and insert sequence determination were appropriately carried out. They show that a single intact copy of the insert is present per haploid genome. In addition no vector backbone sequence was detected. Flanking maize genomic sequences were also determined. Bioinformatic analyses showed a 33 bp deletion at insertion site. These analyses appropriately showed that the insert is located in a non-coding repetitive region of the maize genome. Flanking regions were also

shown to be devoided (in all possible reading frames) of any sequence potentially coding for known allergens and toxins.

Comment 2

Appendices 6 and 8 **(from CBI)** describe several primer sets to amplify the insert and flanking sequences. Hereby a reference is made to New (2010) **(from CBI)**. It could be of interest to mention the technique used to amplify the flanking regions, since the sequences of these regions are then used to design some of the mentioned primers.

Further: no comments.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

Different plant organs at different growth stages were collected at four locations in USA. Expression analysis of both insert encoded proteins was adequately carried out using two ELISA.

Comment 2

No comments

Comment 3

The information provided in the application is sufficient.

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

The information provided in the application is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

Provided information support the conclusion that the insert is stably inherited and expressed.

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

Comments/Questions of the expert(s)

Comment 1

It has been shown that fragments of inserted DNA in foods (as well of non GM-food DNA) can be found in animal tissues and in milk (Mazza et al., 2005). Physiological activity of oligodeoxi-nucleotides has been shown (e.g. Dorn et al., 2007).
Question: Are there data showing that potential fragments of the inserted new chimeric gene ecri3.1Ab, which could occur in tissues of animals and man after consuming 5307 maize, are harmless?

Comment 2

No comment

Comment 3

The information provided in the application is sufficient.

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

The “5307 maize” used for comparative assessment and for the feeding studies were done with one hybrid (NP 2171/NP2460(5307). (Other hybrids were used in the 4-generation stability study; Annex 25). The request for authorization is for “5307 maize”).
Question: Can biological equivalence illustrated for NP 2171/NP2460(5307) be assumed for other 5307-hybrids than the one tested? (In my opinion, the few significant differences noted in composition between the genetically modified maize and its near-isogenic control are not biologically important).

Comment 2

There seem to be no statistically significant differences between the 5307 maize and its nontransgenic comparator for the secondary metabolite and anti-nutrient composition in grain.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

See above

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

Forage and grain were analysed. The compounds analysed for comparative purposes were those suggested by OECD 2002.
In my opinion it is important to show that the potential for growth of mycotoxin producing fungi is not affected by the genetic transformation. Maize is a major food contributing to aflatoxin exposure which contributes to hepatocellular cancer in adults and to poorer growth in children and animals. Aflatoxin exposure occurs in European populations (Khlangwiset et al., 2011) (the finding that in the 3 different broiler study diets mycotoxins were absent (Annex 38, page 20) does not exclude the possibility that maize 5307 hybrids have other potential for fungi growth than other maize varieties).

Additional comment from the SBB:
In annex 38 of the application an Event 5307 hybrid is compared with a nontransgenic near isogenic maize – both did'nt show traces of mycotoxins.
The detection of mycotoxins is not required by the OECD guidance.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

Not applicable and the information provided in the application is sufficient.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

The information provided in the application is sufficient.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

The information provided in the application is sufficient.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

The information provided in the application is sufficient.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

Two studies on mice are presented. In one study the number of replicates seem to be too small in order to detect differences with respect to body weight and feed intake. However, in the second study this is only the case for feed intake. Both the CV and the % difference to be detected are much higher, probably to be explained by the much larger standard deviation for feed intake in the male animals, which gender difference cannot be explained by the classical physiological mechanisms.

Comment 2

It has been claimed that animal feeding experiments longer than 3 months are required in other to demonstrate possible subchronic toxicity of GMOs. Furthermore, it has been suggested that metabolomic studies should be performed on GMO and their non-GM counterparts (de Vendomois et

al., 2010). Slight differences in metabolic profiles between GMOs and their counterparts have been demonstrated (Adel-Patient et al., 2011).

Comment 3

Appendices 33 and 34 deal with the acute toxicity of eCry3.1Ab and PMI, respectively. Has the combined toxicological effect of eCry3.1Ab and PMI ever been tested, since both proteins were expressed in the genetically modified 5307 maize?

There has been an extended assessment of the toxicity of eCry3.1Ab and PMI, based on a series of studies:

- Acute oral mouse toxicity studies
- Molecular and biochemical characterisation
- Searches for homology to known toxins that affect human or animal
- Resistance to proteolytic degradation
- Effect of temperature on eCry3.1Ab insecticidal activity

There was no detrimental effect of eCry3.1Ab and PMI based on these tests, which is in favour of event 5307 maize. However, some of these tests have some restrictions:

- The mouse toxicity studies (Appendices 33 and 34) were conducted using only 5 males and 5 females, which is a limited number of animals (Séralini et al., 2009). OECD (1998) recommends to use at least 10 females and 10 males.
- Only an acute oral toxicity study was performed. Why was no 90-d subchronic toxicity study conducted according to OECD (1998), FAO/WHO (2000) and EU (2001)?
- Toxicity studies with mCry1Ab, used for the formation of the chimeric protein eCry3.1Ab involved in event 5307 maize, showed controversial results for some hematology values and blood chemistry (Hammond et al., 2006).There were a few statistically significant differences between the MON 810 and the control groups after a 90-day rat toxicity test.
- The test substances were produced using recombinant E. coli; Freese and Schubert (2004) mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins.
- Even if GM proteins are rapidly degraded in vitro, Cry1Ab was not fully digested in vivo in pigs (Chowdhury et al., 2003), in calves (Chowdhury et al., 2004) and in cows (Paul et al., 2010).
- eCry3.1Ab is inactivated upon heating at temperatures of 95ºC and above, but such a high temperature is mostly not applied in animal feed manufacturing.

Additional comment from the SBB:
The EFSA "Guidance for risk assessment of food and feed from genetically modified plants" (EFSA Journal 2011; 9(5):2150) states that: "In cases where molecular, compositional, phenotypic, agronomic and other analyses have demonstrated equivalence between the GM plant and derived food and feed and its comparator, except for the inserted trait(s), and have not indicated unintended effects, the performance of animal feeding trials with rodents or other (target) animal species (e.g. broilers) is of little additional value if any, and is therefore not deemed necessary on a routine basis. »

Comment 4

The concentration of the protein eCry3.1Ab was detectable or quantifiable in all tissues analyzed while the concentration of PMI was detectable or quantifiable in most of the 5307 maize tissues analyzed. The amounts seem to be comparable with earlier notifications.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

Petersen et al. (2005) reported that the selectable marker PMI has little risk in terms of biosafety.

Comment 3

a) Degradation of the eCry3.1Ab protein in simulated gastric fluid (Appendix 39).

The eCry3.1Ab protein was readily degraded in SGF.

b) Degradation of the eCry3.1Ab protein in simulated intestinal fluid ().

Not performed.

c) eCry3.1Ab: Acute Oral Toxicity Study in Mice (Korgaonkar, 2009 (Appendix 33)).

Group Number	Test Substance	Dose Level (mg/kg/day)[b]	Dose Volume (mL/kg)	Number of Animals[c] Males	Females
1	Vehicle[a]	0	0	5	5
2	ECRY3.1AB-0208	2000	200	5	5

[a] = Vehicle was 0.5% carboxymethylcellulose
[b] = Dose level and concentration refer to concentration of active ingredient (adjusted by a factor of 1.116 to account for active ingredient purity in the test substance). Therefore, the dose level of the test substance was 2232 mg/kg and the concentration of the test substance in the dosing formulation was 223.2 mg/mL.
[c] = All animals/sex/group were euthanized following 14 days of observation.

Survival: All animals survived to the scheduled necropsy.

Clinical Observations: There were no test substance-related clinical observations.

Body Weights: Body weights were unaffected by test substance administration.

Food Consumption: Statistically significantly higher mean food consumption was noted for the 2000 mg/kg group males on study days 8-9 and 12-13. This difference in food consumption was considered

incidental and not related to test substance administration because the magnitude of the change was small and no changes in food consumption were noted for the remaining study intervals.

Gross Necropsy: Review of the gross necropsy observations revealed no observations that were considered to be associated with administration of the test substance.

Microscopic examination: All histologic changes were considered to be incidental findings or related to some aspect of experimental manipulation other than administration of the test substance. There was no test substance-related alteration in the prevalence, severity or histologic character of those incidental tissue alterations.

Conclusion:
There were no adverse effects of the eCry3.1Ab enzyme when administered by oral gavage at a dose of 2000 mg/kg in male and female mice.

e) eCry3.1Ab: Assessment of Amino Acid Sequence Homology with Known Toxins (McClain, 2011 (appendix 14)).

These results support the conclusion that the eCry3.1Ab amino acid sequence shows no significant similarity with any known or putative toxins that affect human or mammalian health.

f) Degradation of the PMI protein in simulated gastric fluid (Appendix 41).

PMI was rapidly degraded in SGF. No intact PMI or degradation products were visible on the Western blot following one minute of exposure to SGF.

g) Degradation of the PMI protein in simulated intestinal fluid ().

Not performed.

h) PMI: Acute Oral Toxicity Study in Mice (Korgaonkar, 2009 (Appendix 34)).

Group Number	Group Name	Dose Level (mg/kg/day) [b]	Dose Volume (mL/kg)	Number of Animals [c]	
				Males	Females
1	Control [a]	0	0	5	5
2	PMI-0105	2000	200	5	5

[a] = The control group was administered deionized water (the vehicle).
[b] = Formulations were adjusted by a factor of 1.117 to account for test substance purity. Therefore, the dose level of the test substance was 2234 mg/kg and the concentration of the test substance in the dosing formulation was 223.4 mg/mL.
[c] = All animals/sex/group were euthanized following 14 days of observation.

Survival: All animals survived to the scheduled necropsy.

Clinical Observations: There were no test substance-related clinical observations.

Body Weights: Body weights were unaffected by test substance administration.

Food Consumption: Food consumption was unaffected by test substance administration.

Hematology: There were no statistically significant differences for any of the hematology parameters when the control and test substance treated groups were compared.

Serum Chemistry: Statistically significantly higher urea nitrogen (males only), alkaline phosphatase (males only) and alanine aminotransferase levels (females only) were noted in the 2000 mg/kg group compared with controls, and were considered test substance-related.

The changes in the urea nitrogen levels were not considered adverse as the changes were of low magnitude and mean levels were within WIL Historical Control Range except for 1 male. In addition, this difference was not noted in the 2000 mg/kg group females. There were no histopathological correlates in the kidney.

The changes in the alkaline phosphatase (ALP) and alanine aminotransferase (ALT) levels were not considered adverse as the changes were of low magnitude and the group mean and individual animal values for ALP and ALT for the 2000 mg/kg group males and females were all within WIL Historical control ranges. There were no histopathological correlates in the liver.

Comments: The creatinine serumlevel does not seem to be significantly effected. So problems with the urea nitrogen – if there are – should be attributed to the liver.

Organ weights

For males, the mean absolute, relative-to-body, and relative-to-brain weights for the testes and the epididymides were statistically significantly lower than for the 2000 mg/kg group when compared with the weights of the control group, and were considered test substance related. However, there were no distinct histologic correlates for weight changes in either organ, and all organ weight differences were within WIL Historical control ranges.
For females, the mean absolute, relative-to-body, and relative-to-brain adrenal weights were statistically significantly higher for the 2000 mg/kg group when compared with the weights of the control group, and were considered test substance-related. However, there were no distinct histopathological correlates, and an opposite trend was noted in the 2000 mg/kg group males, where adrenal weights were slightly lower (but not statistically significant) than the control group.

Gross Necropsy: Review of the gross necropsy observations revealed no observations that were considered to be associated with administration of the test substance.

Microscopic examination:

There were no test substance-related microscopic findings.
One 2000 mg/kg group female (no. 1667) displayed minimal cytoplasmic vacuolation of renal tubular cells within the outer stripe of the outer medulla. Although this singular finding was most consistent with tissue processing artifact, a test substance relationship could not be entirely ruled out.
All additional microscopic findings were consistent with common, spontaneous alterations in

laboratory mice, or changes associated with some aspect of experimental manipulation other than administration of the test substance.

Remarks: One female showed a mild chronic inflammation of the liver. No such observation for the male group.

In the dossiers EFSA/GMO/DE/66 and 67, no effects were observed in the acute test for PMI at a dosing regimen of 3030 mg/kg.

Conclusion: Please provide further evidence that the PMI protein is non-toxic to the liver.

i) PMI: Assessment of Amino Acid Sequence Homology with Known Toxins (Harper, 2011 (Appendix 17)).

These results support the conclusion that the PMI amino acid sequence shows no significant similarity with any known or putative toxins.

Comment rephrased by the coordinator:
Remarks: One female showed a mild chronic inflammation of the liver. No such observation for the male group. Please provide further evidence that the PMI protein is non-toxic to the liver. If no further evidence that the PMI protein is non-toxic to the liver is provided, then a 90-day rat feeding study should be performed.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

As no metabolomic studies were done (these are not required), one can never be sure if new constituents are formed.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

Of the five natural food components (5/55) that were found to be consistently different in the trials carried out in material of the 2008 and 2009, four of them were fatty acids (Part I, page 43).
Question: Can an explanation be given for these unintended effects?

Comment 2

The information provided in the application is sufficient.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

Only one study was performed (49 day poultry study) to evaluate the wholesomness of the whole food/feed (Appendix 38). It has been suggested that longer studies are required in at least three species, preferential multi-generational, to obtain valid data on toxic effects of GM foods/feeds (de Vendomois et al., 2009).

Additional comment from SBB:
See comment on animal feeding trials under point D.7.8.

Comment 2

a) 49-day feeding study in broiler chickens (Brake, 2010 (Appendix 38).
Starter, grower, and finisher diets were prepared using each source of maize grain and were formulated to meet the nutritional requirements of broilers for each stage of growth, with the maize content of the diets ranging from approximately 52% to 64% by weight. These diets were fed in succession over 49 days.

Observations

The overall survival was approximately 98% for both males and females averaged together at the end of the study. There were no maize grain source effects on survival at any time. In addition, there were no maize grain source-by-sex interactions at any time.

Performance

Performance over the 42-day test period of broilers fed diets containing soybean meal produced from MON 87708 was not different (P ≥ 0.05) than that of broilers fed diets formulated with control soybean meal produced from conventional soybean with similar background genetics to that of MON 87708. Performance over the 42-day test period was also not different (P ≥ 0.05) for birds fed diets containing soybean meal produced from MON 87708 compared to the population of birds fed diets containing conventional control or reference soybean meal.

Body Weights

There were no statistically significant differences in body weight among broilers fed 5307 diets, nontransgenic diets, or NCSU 2007 diets at any time. Furthermore, there were no maize grain source-by-sex interactions for body weight.

Feed Consumption

There were no overall differences in feed consumption over the entire length of the experiment (days 1 to 49). Furthermore, there were no maize grain source-by-sex interactions for feed consumption.

Carcass Measurements

There were no statistically significant differences in the carcass portions (on an absolute weight basis) for males.
Male broilers fed 5307 diets had decreased thigh weights (as a percentage of total body weight) compared with males broilers fed nontransgenic diets, but were no different than the males consuming the NCSU 2007 control diets. Also there were no differences noted in the other carcass parts including: fat pads, drums, wings, and *pectoralis major* and *minor* muscles.
There were no statistically significant differences in the carcass portions (on an absolute basis) for females. Female broilers fed 5307 diets had decreased thigh and *pectoralis minor* weights (as a percentage of total body weight) compared with female broilers fed the nontransgenic and NCSU 2007 diets. This effect was not significant for the absolute thigh and *pectoralis minor* weights. Also there were no differences noted in the other carcass parts including: fat pads, drums, wings, and *pectoralis minor* muscles There were no maize source-by-sex interactions for carcass yield.

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

b) 90-Day rat feeding study ().

If no further evidence that the PMI protein is non-toxic to the liver is provided, then a 90-day rat feeding study should be performed.

Comment 2

The information provided in the application is sufficient.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

In the text (Part I, page 60), the existence of occupational allergy to maize dust is mentioned. In some people, oral intake of maize can elicit severe symptoms such as anaphylaxis (Pastorello et al., 2009). A sensitive method to quantify the maize lipid transfer protein has been published recently (Kuppannan et al., 2011).
It is claimed (Part I, page 60) that the EFSA GMO Panel concluded “that it is unlikely that any interactions between newly expressed proteins and metabolic pathways of maize would alter the pattern of expression of endogenous proteins/potential allergens…”. (The reference is EFSA 2010 and not EFSA 2010**c** (Part I, page 60). The latter reference does not appear in the reference list). Although

this statement is very likely true, I suggest that the level of maize lipid transfer protein would be determined in maize 5307 and compared to published values for several maize lines (Kuppannan et al., 2011), considering the life threatening potential of this protein in some people and the availability of suitable methodology.

Comment 2

Cry proteins and *B. thuringiensis* products have a history of safe use. Furthermore, Petersen et al. (2005) reported that the selectable marker PMI has little risk in terms of biosafety.

However, the rapid degraded of GM proteins is not a guarantee for the lack of an allergenic potential in novel foods (Meredith, 2005). Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein. Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain. None of these tests showed a detrimental effect of eCry3.1Ab and PMI, which is in favour of event 5307 maize.

Additional comment from coordinator and SBB:
We have compared the data in this new application with previous applications regarding GM maize containing the selectable marker PMI: this new application do not contain new data of in vitro and/or in vivo tests. However, the bioinformatic data on sequence homology has been updated by comparison with the FARRP AllergenOnline database, version 2010. The results revealed no homology greater than 35% between PMI an any entry in the database.
Why does this dossier say nothing about the 29% homology of PMI with the Hev b13 allergen, as was done in dossiers EFSA/GMO/UK/2005/11, EFSA/GMO/UK/2007/48 and EFSA/GMO/UK/2007/50 ?

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

Compositional analysis has demonstrated that no unexpected alterations in nutrients and other food components have occurred and that no nutritional imbalances were introduced in 5307 maize.

Appendix 38 deals with the evaluation of event 5307 transgenic maize grain in a broiler chicken feeding study. Obviously, one animal performance experiment has been conducted, as far as published in the literature (Sauvé and Brake, 2010), which is an abstract of Appendix 38.
Furthermore, only 180 chickens were fed a diet containing event 5307 Maize. To my knowledge, no other experiments dealing with animal performance have been mentioned.

It is amazing that the moisture content was high in Appendix 38, Table 2 (15.36% for event 5307 maize) compared to Appendix 31, Table 6 (10.13% in 2008, and 13.92% in 2009). Is there any explanation for this discrepancy?

Comment 3

The information provided in the application is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

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

The information provided in the application is sufficient.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

NB: In the fields, the plants do not use the carbohydrates of the soil as a carbon source but atmospheric CO2. In laboratory, carbohydrates are used in the culture media as a carbon source for plant cells.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

Not applicable and the information provided in the application is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

Known maize allergens (Fasoli et al., 2009) were not measured and allergenic reactions to corn in humans have been described (e.g. Venter et al., 1998; Pastorello et al., 2009; Kumar et al., 2010).

Comment 2

eCry3.1Ab and PMI proteins are present in low levels in food so that exposure to these proteins would be minimal. Cry proteins and *B. thuringiensis* products have a history of safe use. Petersen et al. (2005) reported that the selectable marker PMI has little risk in terms of biosafety. Acute oral toxicity studies showed that the oral administration of 2 g eCry3.1Ab or PMI per kg body weight to mice as a single dose did not result in adverse clinical signs.

However, only 10 mice were involved to investigate toxicity, which is a limited number of animals.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

The reported study on broilers seems to be sufficient with respect to number of replications for testing effects on feed conversion ratio and mortality, but not for body weight and feed intake, which is looking rather contradictory.

Comment 2

Allergic reactions to maize have been described in animals (e.g. White DS, 1998; Krishnan et al., 2010).

Comment 3

eCry3.1Ab and PMI proteins are present in low levels in feed so that exposure to these proteins would be minimal. Cry proteins and *B. thuringiensis* products have a history of safe use. Petersen et al. (2005) reported that the selectable marker PMI has little risk in terms of biosafety. Acute oral toxicity studies showed that the oral administration of 2 g eCry3.1Ab or PMI per kg body weight to mice as a single dose did not result in adverse clinical signs. No deleterious effects on bird health were observed in the study of Sauvé and Brake (2010). Only one experiment from one location has been reported.

Consequently, only 10 mice were involved to investigate toxicity, and obviously only one animal performance experiment, involving 180 broiler chickens fed event 5307 maize, has been reported in this dossier.

Comment4

The applicant answers to this point in D.9.6. For animal health the information is sufficient.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment1

See D.9.7 in the application. Not applicable and the information provided in the application is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

Not relevant, at least not for the EU, as it is not the intention to cultivate event 5307 maize in the EU.

Comment 2

See D.9.8 in the application. Not applicable and the information provided in the application is sufficient.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

Not applicable and the information provided in the application is sufficient.

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

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