Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2017_0066

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/NL/2010/89 from Dow AgroScience under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2010/89 was submitted by Dow AgroScience on 11 November 2010 for the marketing of genetically modified maize DAS-40278-9 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003[1]. Maize DAS-40278-9 contains a single insert expressing the aryloxyalkanoate dioxygenase-1 (AAD-1) protein, which confers tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) herbicides.

The application was officially acknowledged by EFSA on 11 March 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). Eight 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 EFSAnet on 8 June 2011.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 26 October 2016 (EFSA Journal 2016;14(12):4633[2]), and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 11 January 2017 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, but none of the experts did so. 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/4633

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Scientific evaluation

1. Environmental risk assessment

The Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of maize DAS-40278-9 seeds (i.e. during transport and/or processing) into the European environment[3] 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 DAS-40278-9, in comparison with its conventional counterpart, do not raise safety concerns.

3.2. Assessment of toxicity

The Biosafety Advisory Council notes that the 28-day toxicity study was not fully compliant with the EFSA guidance (the EFSA recommendation to use a higher number of animals was not fulfilled for the examination of the haematological, clinical chemistry and coagulation parameters).
Therefore, the Biosafety Advisory Council is of the opinion that no conclusion can be drawn about the toxicological safety for humans and animals of this GM maize.

3.3. Assessment of allergenicity

The potential allergenicity of the newly expressed AAD-1 protein has been assessed in the context of this application and no concerns in relation to allergenicity were identified.

With regard to the allergenicity of the whole GM plant, to date maize is not considered to be a common allergenic food. Based on the available information, the Biosafety Council considers that there is no evidence that overall allergenicity of maize DAS-40278-9 is changed as a result of the genetic modification.

3.4. Nutritional value

Based on compositional data the Biosafety Advisory Council agrees with the EFSA GMO panel that the nutritional value of food and feed derived from maize DAS-40278-9 is not expected to differ from that of food and feed derived from non-GM maize varieties.

4. Monitoring

Since the allergenicity of the whole GM maize has not been fully assessed, it is recommended to take up monitoring of allergenicity as part of the general surveillance.

[3] As the application doesn't include the cultivation of the GM crop in the EU, a full environmental assessment is not required by EFSA procedure and was not performed.

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 shortcomings in the 28-day toxicity study, it is not possible to draw a final conclusion about the food and feed safety of maize DAS 40278-9 in the context of its proposed uses.

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

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

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/NL/2010/89 and *comments submitted on the EFSAnet (ref. BAC_2011_0497).*

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08-06-2011

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Compilation of comments of experts in charge of evaluating the application EFSA/ EFSA/GMO/NL/2010/89 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 23 March 2011
Coordinator: Prof. Dirk Reheul
Experts: Leo Fiems (ILVO), Rony Geers (KUL), Johan Grooten (UGent), André Huyghebaert (UGent), Birgit Mertens (WIV-ISP), Peter Smet (Consultant), Frank Van Breusegem (VIB-UGent), Michel Van Koninckxloo (HEP Hainaut- Condorcet)
Domains of expertise of experts involved: Genome analysis, genetic engineering, human nutrition, animal nutrition, analysis food/feed, substantial equivalence, traceability of alimentary chain, toxicology, general biochemistry, allergology, agronomy, agro-ecology, risk analysis, maize
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **EFSA/GMO/NL/2010/89** concerns an application of the company **Dow AgroScience** for the renewal of the marketing authorisation of the genetically modified **maize DAS 40278-9** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 11 March 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). 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. 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

The genetically modified maize DAS 40278-9 is not likely to cause adverse effects on animal and human health:

- A total of 82 different compositional analyses confirm the substantial equivalence of event DAS-40278-9 and conventional maize
- the Sphingobium herbicidovorans bacterium, used for AAD-1 gene isolation, is an ubiquitous soil organism; Sphingobium herbicidovorans is not known as a human pathogen or producing allergens
- AAD-1 protein has been demonstrated to present a low risk of toxicity; furthermore, heating conditions largely eliminate the enzymatic activity of the AAD-1 protein; therefore, it is unlikely that DAS-40278-9 event will cause adverse effects in humans or animals
- no amino acid sequences were generated with significant similarities with toxic proteins that are harmful to humans and animals

Comment 2

No comments. Maybe in future dossiers the ref. Wright et al. (2010) PNAS could be mentioned together with the current ref. to a parent application (2008).

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

The information provided in the application is sufficient.

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

Comments/Questions of the expert(s)

Comment 1

No comments

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 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

No comments

Comment 2

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

No comments

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

No comments

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 comments

Comment 2

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

No comments

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

No comments

Comment 2

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

Values for anti-nutrients and secondary metabolites seem to be within the literature and reference range.

Comment 2

A traditional approach is followed in the comparative assessment: DAS maize is compared with non-GM control maize with comparable genetic background and with six conventional maize hybrids.
As the actual OECD guidelines are followed, I will only comment on the selection of compounds for the comparative analysis to a limited extend. To my knowledge the OECD guidelines are under review.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

Eight sites were selected in different states of the US for the production of material for further analysis. As mentioned above maize hybrids were included, in addition to the DAS-40278-9 maize and the Non-GM control . Herbicides were applied at the maximum allowable rate.
I have no further comments on the production of material for the assessment, the statistical models used and the baseline used for consideration of natural variations.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

Studies designed to evaluate the AAD-1 protein for allergenic and toxic characteristics were conducted using AAD-1 protein produced by Pseudomonas fluorescens. With regard to microbial protein, Freese and Schubert (2004) mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins. However, the combination of several analyses (safety of the donor organism, homology with known allergens, *in vitro* simulated gastric fluid digestibility), resulting in a holistic, integrative approach, means that the chance for safety risks may be very low.

Comment 2

Compounds analyzed include proximate (ash, fat, moisture, protein, carbohydrates), total dietary fibre, acid detergent fibre, neutral detergent fibre, minerals, amino acids, fatty acids, vitamins, secondary metabolites and anti-nutrients.
This is a traditional approach focusing on natural constituents.

Maize is known to be sensitive to mould attack and presence of particular **mycotoxins**. Information on this issue would be very welcome as samples are produced at different locations and probably variable production conditions.
In terms of food safety there is more concern about the potential presence of mycotoxins than about small amounts of not very powerful anti-nutrients. Information on the question if the technology applied results in any modification in sensitivity to mycotoxin formation would be very welcome.
It is to be admitted that this is not probable but this is also the case for other nutrients and anti-nutrients.

No biologically meaningful differences were found for **proximate** en **fibre** constituents. I agree with this conclusion.

With respect to **minerals,** analysis results include calcium, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium and zinc. No significant differences were found for most minerals. If particular differences were observed, such as for calcium, results were within the range of literature values.

Results for **amino acids** cover the whole range of relevant constituents. After statistical analysis and evaluation of the data, the applicant concludes that no difference is found in most cases. If any significant difference is observed, values are within the range of literature data.

A similar conclusion applies for the data of **fatty acid** analysis. Results cover the whole range of fatty acids even minor fatty acids with short and very long chain length, below detection limit.

Vitamin analysis includes relevant constituents like β-carotene, vit B1, B2, B6, C, E, niacin and folic acid.
The applicant discusses results and concludes that all values are within the range of literature data. This also applies for important constituents like β-carotene and niacin.

It is surprising that no statistical analysis was performed for vit E or tocopherols as most results were below LOQ or limit of quantification. Maize oil is known to be a good sources or tocopherols (natural antoxidants).
No attention is given to other carotenoids like zeaxanthin and lutein, well known natural pigments in maize. In addition to their antioxidant activity they are important in human and animal nutrition. Some of these pigment are related to eye health in humans (age related macular degeneration).

A range of **anti-nutrients** and **secondary metabolites** was also analysed. Data for inositol, furfural, p-coumaric acid, ferulic acid are included as secondary metabolites. Data for anti-nutrients include phytic acid, raffinose and trypsin inhibitor. The applicant formulates as similar conclusion as for previous constituents.

As a **general comment** I would like to emphasise that all data are related to nutrients, secondary metabolites and anti-nutrients. This approach does not cover the whole composition of the product, in this case maize. Contaminants, particularly of environmental origin, are often present.

As mentioned previously there is no information on the effect of mycotoxin formation.
The uptake of heavy metals from soils could be influenced by the applied technology. It is recommended to pay more attention to contaminants in future OECD guidelines.

On the other hand I agree with the general conclusion of the applicant on this part of the dossier.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

Comment 2

No comment

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

Comment 2

No comment

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

Comment 2

The applicant states that DAS-40278-9 maize will be converted into the same food and feed products as conventional maize. No novel production process is envisaged.
It is demonstrated that the AAD-1 protein is rapidly degraded under the processing conditions.
No further questions.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

Results of a calculation of the MOE (margin of exposure) calculation are presented, for a short term intake. The values are significantly higher than 10.000. Values higher than 10.000 are of no concern in terms of food safety.

Values for MOE upon repeat dose assessment are also calculated. The most recent models of EFSA are applied. This approach includes specific diets by subpopulations. Values obtained are > 4500.

I agree with the conclusions with respect to intake by humans.

Comment 2

The estimated acute and chronic exposures to AAD-1 protein via the modified maize indeed are too low to expect toxic side-effects from the AAD-1 protein. They are however still in a range that allows for allergenicity especially upon chronic, long term exposure as can be anticipated for food.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

Because

- Sphingobium herbicidovorans is not known as a human pathogen or producing allergens
- heating protein solutions virtually eliminated the enzymatic activity of the AAD-1 protein
- the AAD-1 protein expressed in maize event DAS-40278-9 contains no significant sequence similarity with any known toxic protein that is harmful to humans or animals
- acute oral toxicity study in mice did not indicate any nutritional effect or safety concern for DAS-40278-9

toxic effects are hardly expected.

Comment 2

Allergenicity is strongly associated with intrinsic enzymatic activity. AAD-1 being inserted in maize for its enzymatic activity has as a result an increased risk of acting as allergen. Therefore, conditions promoting loss of enzymatic activity are highly relevant for assessing potential toxicity but also potential allergenicity.

Heating of the protein at temperatures of 50°C and higher resulted in a rapid loss of AAD-1 enzymatic activity. However, it remains unclear to what extend and at what rate heating AAD-1 at body temperature induces loss of enzymatic activity, a feature important for assessing potential allergenicity.

Comment 3

Two toxicity studies are reported, i.e. Wiescinski and Golden (2007) and Cleveland (2010), but the data do not allow an evaluation of the validity of the experimental design.

Two feeding trials are reported, i.e. Fletcher (2010) with broilers, and Thomas and Marshall (2010) with mice. The broiler trial is designed so that statistical analysis of the data has sufficient power, which is not the case for the mice trial.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

<u>Protein used for safety assessment</u>

The inserted sequence in DAS-40278-9 maize encodes for a new protein, e.g. the aryloxyalkanoate dioxygenase (AAD-1) enzyme. Given the low expression level of AAD-1 in DAS-40278-9 maize, the applicant decided to use a microbial analogue of the AAD-1 protein, produced in *Pseudomonas fluorescens,* for safety testing. Both bacterially produced AAD-1 potein and plant-expressed AAD-1 isolated from stalk tissue of DAS-40278-9 maize displayed a ~33 kDa band on Western blots. Glycosylation was analysed after SDS PAGE using a commercial staining kit. The results demonstrated that the plant-expressed AAD-1 protein was not glycosylated. Moreover, MALDI-TOF mass spectrometry performed on trypsin-digests of the AAD-1 proteins confirmed that the amino acid sequence of the plant-derived AAD-1 protein was equivalent to the *P. fluorescens* protein. The peptide sequence of both proteins was also similar as demonstrated by tandem mass spectrometry. Based on these data, AAD-1 derived from *P. fluorescens* was considered as an appropriate substitute of the plant-expressed AAD-1 isolated from stalk tissue of DAS-40278-9 maize for safety testing.

<u>Toxicological assessment of the novel protein in DAS-40278-9 maize</u>

The newly introduced gene in DAS-40278-9 maize is derived from the gram-negative soil bacterium *Sphingobium herbicidovorans*. No reports of *S. herbicidovorans* being implicated as a human pathogen or as a source of allergens are available. Information on the primary sequence, molecular weight, thermal stability, substrate specificity and possible reaction products of the AAD-1 enzyme is provided by the applicant.

Bioinformatic searches

Searches for amino acid sequence homology of the AAD-1 protein expressed in DAS-40278-9 maize with amino acid sequences of toxic proteins indicated no significant sequence similarity to any known proteins that are harmful to humans or animals. Additional assessment did not reveal any similarities of the insert and its flanking regions to known toxins that could cause adverse effects in humans or animals.

Acute oral toxicity

An acute oral study was performed in Crl:CD1 (ICR) mice dosed with 2000 mg AAD-1 protein/kg body weight. No effects related to administration of AAD-1 protein were noted on clinical observations, gross necropsy and mortality 14 days after the administration.

28-day repeat study in rats

The applicant provided a 28-day repeated dose feeding study in which groups of 5 Crl:CD1 (ICR) mice of each sex were given a diet formulated to supply 0, 0.452, 4.523 or 45.23 mg (1000x greater than the estimated "worst case" daily human consumption) AAD-1 protein per kilogram body weight per day. An additional group of five male and five female mice received diets containing bovine serum albumin (BSA) formulated to supply 45.23 mg/kg bw/day to serve as a protein control group. There were no treatment-related effects on any of the studied parameters including cage-side and clinical observations, ophthalmic examinations, body weights, feed consumption, haematology, clinical chemistry, and gross and histopathologic examinations.

Comments

- Temperature and pH range for optimal activity of the enzyme were not specified.
- Normally the rat is the preferred rodent species to study acute and repeated dose toxicity. If another rodent species is used, a detailed justification should be given.

Remark from the SBB

For the toxicity studies the EFSA guidance refers to tests with rodents but do not specify that the rat should be the preferred species.

Comment 2

a) Degradation of the AAD-1 protein in simulated gastric fluid (Embrey and Korjagin, 2008).

AAD-1 protein is readily digested by pepsin (not detectable at 30 seconds) in simulated gastric fluid as demonstrated by both SDS-PAGE and western blot analyses.

b) Degradation of the AAD-1 protein in simulated intestinal fluid ().

Not performed.

c) AAD-1: Acute Oral Toxicity Study in Mice (Wiescinski and Golden, 2007).

Mortality

All animals survived the treatment period.

Clinical Observations
All animals appeared normal throughout the study.

Body Weights
Eight out of the ten animals gained or maintained weight by test day 2. All animals gained weight by study termination on test day 15.

Necropsy
There were no treatment-related gross pathological observations.

CONCLUSIONS
No signs of acute oral toxicity in mice after administration of 2000 mg/kg AAD-1.

d) AAD-1: Repeated dose oral toxicity (28-day feeding) study in mice (Thomas and Marshall, 2010).

Dose Levels (mg AAD-1 protein/kg bw/day)*	No. of Mice/Sex/Dose
0	5
45.23 mg BSA /kg bw/day (protein control)	5
0.452	5
4.523	5
45.23	5
TOTAL	50

* Dose levels were corrected for purity.

Mortality
All animals survived the full duration of the test period.

Detailed Clinical and Cage-side Observations
There were no treatment-related in-life observations for any of the treatment groups.

Ophthalmology
Examinations performed on all animals prior to termination (day 28) revealed no treatment-related effects in the eyes.

Body Weights/Body Weight Gain

Body weights and body weight gains of males of all dose groups given AAD-1 protein were comparable to the BSA-treated and untreated controls.

Mean body weight and body weight gain of low-dose females (0.452 mkd AAD-1 group) were lower than those of the respective BSA-treated control on day 29.

Sex	Females				
Treatment groups	Controls		AAD-1		
Dose (mkd)	0	45.23 (BSA)	0.452	4.523	45.23
Body Weight (g)	28.1	26.5	25.0	27.2	26.9
Body Weight Gain (g)	5.9	4.1	3.0	4.7	5.0

Seems to be of no concern due to the absence of a dose-response relationship.

Feed Consumption
There were no statistically identified or treatment-related differences in the amount of feed consumed by either sex of the AAD-1 protein-treated group throughout the study compared to the respective BSA-treated controls.

Hematology
There were no treatment-related or statistically identified differences between untreated and BSA-treated controls of either sex, or between all dose-groups of either sex given AAD-1 protein as compared to their respective BSA-treated controls.

Clinical Chemistry
There were no statistically identified differences between the BSA-treated controls and untreated controls of either sex. There were no treatment-related or statistically identified differences between AAD-1 protein treated groups of either sex as compared to their respective BSA-treated controls.

Anatomic Pathology
Organ Weight
There were no statistically identified or treatment-related effects in the organ weights for males and females given AAD-1 protein at any dose level as compared to the BSA-treated controls.

Gross Pathology
There were no treatment-related gross pathologic observations. All gross pathologic observations were considered spontaneous alterations, unassociated with exposure to AAD-1 protein.

Examination of each of the alterations revealed **no** dose-response relationship.

Histopathology
There were no treatment-related histopathologic observations. All observations were considered spontaneous changes unassociated with exposure to AAD-1 protein.

Some statistical significant differences compared to the control were observed, but in none of these cases, a dose-response relationship could be established.

Conclusion: the 28-day repeated feeding study revealed no signs of toxicity as a result of the genetic modification.

e) AAD-1: Assessment of Amino Acid Sequence Homology with Known Toxins (Song, 2010b)

The AAD-1 protein contains no significant sequence similarity with any known toxic protein.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

Since no new constituents other than the AAD-1 protein were expressed in DAS-40278-9 maize, 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

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

42-day feeding study using boiler chickens
Five treatment groups of 120 birds (12 replicates of ten birds each), balanced by sex, received diets prepared with either transgenic maize (DAS-40278-9), non-transgenic maize or meal made from standard commercially available maize. Results from the study showed that DAS-40278-9 maize was nutritionally similar to feed prepared with non-transgenic maize.

Comment
The applicant should justify why a 90-day rodent feeding study with the whole GM food was not performed.

Remark from the SBB
The Biosafety Council agrees with EFSA that a 90 day feeding study is not required when the compositional analysis has demonstrated the substantial equivalence between the GM and non-GM plant.

Comment 2

a) 42-day feeding study in broiler chickens (Fletcher, 2010).
Observations
No behavioral differences were noted in broilers between the various groups.

Body Weights
Statistical analyses of individual body weights at initiation and termination (day 42) revealed no significant differences among the five treatment groups.
Statistical analyses of weight gain among the five treatment groups over the entire study (42 days) revealed no significant differences.

Feed Consumption
Statistical analyses of the average daily feed consumption over the entire study (42 days) among the five treatment groups revealed no significant differences.

Feed Conversion
Statistical analysis of feed conversion for phase 1, phase 1 and 2 combined or phases 1, 2 and 3 combined (Feed:Gain ratio) revealed no significant differences (P≥ 0.05) among the five treatment groups.

Carcass Measurements
Statistical analyses of the carcass trait data of broilers fed the genetically modified (transgenic) corn versus those fed the genetically similar (non-transgenic) corn revealed no significant differences.
Statistical differences were noted in carcass data or weights of cuts between the genetically modified (transgenic) corn and the commercial corn varieties. These differences were limited to two of the three commercial groups and did not occur between the genetically modified (transgenic) group and the genetically similar (non-transgenic) group.

Conclusion: No biologically relevant effects on body weights, feed consumption, feed conversion or carcass measurements.

b) 90-Day rat feeding study ().

No further testing is needed.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

A rapid degradation of the aad-1protein occurred in simulated gastric fluid (SGF); however, a rapid in vitro digestion is not a guarantee for the lack of an allergenic potential in novel foods (Meredith, 2005). 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, and Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein. However, the combination of several analyses (safety of the donor organism, homology with known allergens, *in vitro* simulated gastric fluid digestibility), resulting in a holistic, integrative approach, means that the chance for allergenic reactions may be very low.

Comment 2

The source of the trait, the lack of amino acid sequence similarity with known allergens and the rapid *in vitro* digestibility in simulated gastric fluid all point to a lack of AAD-1 allergenicity. However, other traits of the AAD-1 protein raise some caution with this conclusion.

The protein is rather small (33 kDa) and has intrinsic enzymatic activity. Both characteristics may promote allergenicity. It is insufficiently documented to what extend AAD-1 enzymatic activity is resistant/sensitive to body temperature of 37°C. Also the data shown on *in vitro* digestibility (figure 36) raise some caution. The SDS-PAGE analysis was apparently run at a rather low sensitivity, making it difficult to detect the AAD-1 band when below 10% of the input. Also the expected AAD-1 breakdown bands are not visible on the gel. Furthermore, the more sensitive Western blot does not allow concluding that the AAD-1 protein was readily digested. The acid treatment of the protein will undoubtedly compromise its recognition by the polyclonal antibody and hence may be responsible for the absence of visible AAD-1 bands in the acid/pepsin treated samples. An additional control consisting of treatment only with acid (no pepsin) is needed to draw a firm conclusion on digestibility.

A limited set of animal data is available from the tox studies. From this data set, differential white blood cell counts from mice fed for 29 days with increasing doses of AAD-1 are helpful to determine whether some level of sensitization occurred in these mice. The data from this study (ID 091026) are not alarming but again do not allow drawing a firm conclusion. The reason for this is a clear tendency towards higher eosinophil blood counts in the male groups treated with low to intermediate doses of AAD-1. Although these higher counts are not statistically significant, they call for a repeat experiment on larger groups of mice. Furthermore, serum samples from these mice should be analyzed by ELISA for the presence of elevated antibody (IgG, IgE) titres to AAD-1.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

A total of 82 different compositional analyses confirm the substantial equivalence of event DAS-40278-9 and conventional maize (Herman et al., 2010). A poultry feeding study confirmed the nutritional equivalence of DAS-40278-9 maize with its non-GM commercial maize equivalent.

Table 28 (P.125 of the Technical Dossier) does not present weight gain and feed conversion efficiencies, as mentioned in the title.

Comment 2

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

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

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.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

This topic is not of relevance, as the application of DAS-40278-9 maize does not intend to cultivate the maize crop in the EU.

Comment 2

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

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

There is little chance that event AAD-1 will exert a detrimental effect on human health (see D.7.8 Toxicology).

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

see comment 3 under D.7.8.

Comment 2

There is little chance that event AAD-1 will exert a detrimental effect on animal health (see D.7.8 Toxicology) .

Comment 3

The information provided in the application is sufficient.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

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

The information provided in the application is sufficient. This maize will not be cultivated in EU.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

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

In the summary (and not in Part I), we can read :
"The general surveillance information reported to and collected by the authorization holder from the European trade associations or other sources will be analysed for its relevance. Where information indicates the possibility of an unanticipated adverse effect, the authorisation holder will immediately investigate to determine and confirm whether a significant correlation between the effect and DAS-40278-9 maize grain can be established. If the investigation establishes that DAS-40278-9 maize grain were present when the adverse effect was identified, and confirms that DAS-40278-9 maize grain is the cause of the adverse effect, the authorization holder will immediately inform the European Commission, as described in Section D.11.5. »
Comment: Analysis of data from general surveillance should be entrusted to an independent centre rather than to the authorization holder.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

References

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Herman, R.A., Storer, N.P., Gao, Y. 2006. Digestion assays in allergenicity assessment of transgenic proteins. Environ. Health Perspect. 114: 1154-1157.

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