SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-UK-2008-53) for the placing on the market of herbicide tolerant genetically modified maize 98140 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Pioneer Overseas Corporation[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2, 3]

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ABSTRACT

Maize 98140 contains a single insert consisting of the *gat4621* and the *Zm-hra* expression cassettes, providing herbicide tolerance. Bioinformatic analyses and genetic stability studies did not raise safety issues. The levels of the GAT and Zm-HRA protein in maize 98140 have been sufficiently analysed. The minimum standards for the design of field trials, set out in the EFSA GMO Panel guidance document, were not met. Therefore the EFSA GMO Panel was not in a position to conclude on the comparative assessment of the compositional, agronomic and phenotypic characteristics, on the basis of the data provided. In the absence of conclusions on the comparative assessment of composition, the risk assessment was restricted to the newly expressed proteins and to specific metabolites resulting from the acetylase activity of the GAT4621 protein. The EFSA GMO Panel has identified a gap in the data on the agronomic and phenotypic characterisation of GM maize 98140 and considers that uncertainty over these characteristics remains. However, considering the scope of this application, the available data and the poor survival capacity of maize outside cultivated land, the EFSA GMO Panel concluded that there is very little likelihood of environmental effects due to the accidental release into the environment of viable grains from maize 98140. Considering its intended use as food and feed, interactions with the biotic and abiotic environment were not considered to be an issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from maize 98140 to bacteria have not been identified. The monitoring plan and reporting intervals were in line with the intended uses of maize 98140.

KEY WORDS

GMO, 98140 maize, food and feed safety, environment, import and processing, Regulation (EC) No 1829/2003, GAT, Zm-HRA, *N*-acetylated amino acids, NAA, NAG.

[1] On request from the Competent Authority of the United Kingdom for application (EFSA-GMO-UK-2008-53) submitted by Pioneer Overseas Corporation, Question No EFSA-Q-2008-301, adopted on 7 March 2013.

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SUMMARY

Following the submission of an application (Reference EFSA-GMO-UK-2008-53) under Regulation (EC) No 1829/2003 from Pioneer Overseas Corporation, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of the herbicide-tolerant genetically modified (GM) maize 98140 (Unique Identifier DP-Ø9814Ø-6) for import and processing for food and feed uses. Maize 98140 was developed to provide tolerance to glyphosate and acetolactate synthase (ALS)-inhibiting herbicides (such as chlorimuron and thifensulfuron).

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-UK-2008-53, additional information provided by the applicant (Pioneer Overseas Corporation) and the scientific comments submitted by the Member States. The scope of application EFSA-GMO-UK-2008-53 is for food and feed uses and import and processing of maize 98140 and all derived products, but excludes cultivation in the European Union (EU).

The EFSA GMO Panel evaluated maize 98140 with reference to the intended uses and appropriate principles described in the EFSA GMO Panel guidance documents for the risk assessment of GM plants and derived food and feed. The scientific risk assessment evaluation included molecular characterisation of the inserted DNA and expression of target proteins. A comparative analysis of agronomic traits and composition was undertaken, and the safety of the new proteins, as individual proteins and in combination, the changed levels of natural constituents and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An evaluation of environmental impacts and the post-market environmental monitoring plan were undertaken.

Maize 98140 has been genetically modified to express the GAT4621 and Zm-HRA proteins. The GAT4621 protein is a glyphosate acetyltransferase (GAT), encoded by an optimised form of the *gat4621* gene from *Bacillus licheniformis*, which confers tolerance to glyphosate herbicides. The Zm-HRA protein is an acetolactate synthase (ALS), encoded by an optimized form of the endogenous *als* gene from *Zea mays*, which confers tolerance to ALS-inhibiting herbicides, such as chlorimuron and thifensulfuron.

The molecular characterisation data establish that the genetically modified maize 98140 contains a single insert consisting of the *gat4621* and the *Zm-hra* expression cassettes. No other parts of the plasmid used for transformation are present in maize 98140. Bioinformatic analyses and genetic stability studies did not raise safety issues. The levels of the GAT and HRA protein in maize 98140 have been sufficiently analysed.

The EFSA GMO Panel considered the compositional, agronomic and phenotypic data supplied in the light of the field trial design. In this application the minimum standards for the design of field trials set out in the EFSA GMO Panel guidance document were not met. Therefore, the EFSA GMO Panel cannot conclude on the comparative assessment of the compositional, agronomic and phenotypic characteristics, on the basis of the data provided. In the absence of conclusions on the comparative assessment of composition, the assessment was restricted to the newly expressed proteins and to specific metabolites resulting from the acetylase activity of the GAT4621 protein. No safety concerns were identified for the newly expressed protein Zm-HRA ALS, based on the widespread occurrence of ALS enzymes in nature and as part of the human and animal diet, lack of known toxicity to humans or animals and lack of similarity to known toxins in bioinformatics analyses. The GAT4621 protein did not show significant similarity to known toxins in bioinformatics analyses either. However, the repeated-dose (28-day) oral toxicity study provided using mice fed with GAT4621 was not performed in accordance with the respective OECD Guideline because not all relevant parameters (i.e. haematology and coagulation) were analysed. Therefore, the EFSA GMO Panel cannot conclude on the safety of the newly expressed GAT4621 protein. The elevated levels of the natural constituents *N*-acetyl-aspartic acid (NAA) and *N*-acetyl glutamic acid (NAG) in maize 98140 were also assessed. The calculated margins between the estimated intakes of NAA and NAG and the highest doses administered without observed effects in the available toxicological studies in animals are considered

sufficient. Moreover, the estimated intakes of NAA and NAG are small in comparison with the habitual dietary intakes of aspartic acid and glutamic acid, respectively. The EFSA GMO Panel is of the opinion that the higher levels of NAA and NAG as such would not raise safety concerns for humans or animals.

Feeding studies in laboratory rats and broiler chickens did not show adverse effects of administering diets containing maize 98140 to these animals compared with diets containing a negative segregant of maize 98140 or conventional maize.

The application EFSA-GMO-UK-2008-53 concerns food and feed uses and import and processing. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of maize 98140. The EFSA GMO Panel has identified a gap in the data on the agronomic and phenotypic characterisation of GM maize 98140 and considers that uncertainty over these characteristics remains. However, considering the scope of this application, the available data and the poor survival capacity of maize outside cultivated land, the EFSA GMO Panel concluded that there is very little likelihood of environmental effects due to the accidental release into the environment of viable grains from maize 98140. Considering its intended use as food and feed, interactions with the biotic and abiotic environment were not considered to be an issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from maize 98140 to bacteria have not been identified. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize 98140. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the monitoring plan.

In the absence of an appropriately performed comparative assessment by the applicant, the EFSA GMO Panel was not in the position to complete its risk assessment on maize 98140 and therefore does not conclude on the safety of maize 98140 compared with its conventional counterpart with respect to potential effects on human and animal health. However, the EFSA GMO Panel concludes that the maize event 98140 is unlikely to have any adverse effect on the environment in the context of its intended uses.

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BACKGROUND

On 15 April 2008, EFSA received from the Competent Authority of the United Kingdom an application (Reference EFSA-GMO-UK-2008-53), for authorisation of the herbicide-tolerant genetically modified maize 98140 (Unique Identifier DP-Ø9814Ø-6), submitted by Pioneer Overseas Corporation within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed (EC, 2003) for food and feed uses, import and processing.

After receiving the application EFSA-GMO-UK-2008-53 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States as well as the European Commission and made the summary of the dossier publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 8 October 2008, the applicant provided EFSA with additional information requested under completeness check (requested on 10 June 2008) and on 12 November 2008 EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Regulation (EC) No 1829/2003 and Directive 2001/18/EC (EC, 2001) following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member State bodies had three months after the date of receipt of the valid application (until 12 February 2009) within which to make their opinion known.

The GMO Panel carried out a scientific assessment of genetically modified (GM) maize 98140 taking into account the appropriate principles described 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, 2006a).

On 23/01/2009, 20/05/2009, 28/05/2010, 26/10/2010, 10/02/2011 and on 24/05/2012 the GMO Panel asked for additional data on maize 98140 (application EFSA-GMO-UK-2008-53). The applicant provided the requested information on 17/03/2009, 06/10/2009, 05/03/2010, 12/07/2010, 22/12/2010, 08/03/2012 and on 13/07/2012. After receipt and assessment of the full data package, the GMO Panel finalised its risk assessment of maize 98140.

The GMO Panel carried out a scientific assessment of the GM maize 98140 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into consideration the scientific comments of the Member States and the additional information provided by the applicant.

In giving its opinion on GM maize 98140 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the receipt of the valid application. As additional information was requested by the EFSA GMO Panel, the time limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, the EFSA opinion shall include a report describing the assessment of the food and feed and stating the reasons for its opinion and the information on which its opinion is based. This document is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the overall opinion in accordance with Articles 6(5) and 18(5).

TERMS OF REFERENCE

The GMO Panel was requested to carry out a scientific assessment of the genetically modified maize 98140 for food and feed uses and import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environments and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)e of Regulation (EC) No 1829/2003.

The EFSA GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol, nor on the proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to GMO risk management.

ASSESSMENT

1. Introduction

The genetically modified (GM) maize 98140 (Unique Identifier DP-Ø9814Ø-6) was assessed with reference to its intended uses, taking account of the appropriate principles described 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, 2006a).

Maize 98140 was developed to express an optimised form of the glyphosate acetyltransferase *(gat4621)* coding sequence from *Bacillus licheniformis*, which confers tolerance to the herbicide glyphosate, and *Zm-hra*, an optimised form of the endogenous acetolactate synthase (*als*) coding sequence from maize (*Zea mays*), which confers tolerance to acetolactate synthase (ALS)-inhibiting herbicides, such as chlorimuron, thifensulfuron and other sulfonylurea herbicides.

The scope of application EFSA-GMO-UK-2008-53 is for food and feed uses and import and processing of maize 98140 and all derived products (e.g. starch, syrups, ethanol, maize oil, flakes, coarse and regular grits, coarse and dusted meal, flour, maize germ meal, maize feed, condensed steep water and maize meal).

The risk assessment presented here is based on the information provided in the application EFSA-GMO-UK-2008-53 submitted in the EU including the additional information from the applicant and the scientific comments that were raised by Member States on this application.

2. Issues raised by Member States

The scientific comments raised by Member States are addressed in Annex G of the EFSA overall opinion and have been considered throughout this EFSA GMO Panel scientific opinion.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs

Maize 98140 has been genetically modified for herbicide tolerance. This was achieved by the introduction of the *gat4621* and the *Zm-hra* coding sequences surrounded by their necessary regulatory components.[4]

- *gat4621* is an optimised form of the glyphosate acetyltransferase *(gat)* coding sequence from *Bacillus licheniformis* that confers tolerance to the herbicide glyphosate. The synthetic *gat4621* coding sequence was obtained after 11 rounds of DNA shuffling using three distinct alleles of the *gat* gene isolated from three different strains of *B. licheniformis*, as well as the introduction of changes via polymerase chain reaction (PCR) (Castle et al., 2004). The glyphosate inhibits the enzyme enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is involved in the biosynthesis of aromatic amino acids. Glyphosate acetyltransferase (GAT) proteins acetylate glyphosate give rise to *N*-acetyl glyphosate, which has no herbicidal activity. The native GAT enzymes are capable of acetylating glyphosate, but at a very slow rate. The GAT4621 protein has 75–78 % identity at the amino acid level to each of the three GAT enzymes from *B licheniformis* from which it was derived, but with a 7 000-fold increased catalytic efficiency. The GAT proteins are members of the GNAT family of *N*-acetyltransferases. GNAT proteins have a number of metabolic functions including detoxification (Dyda et al., 2000). The studies on substrate specificity of GAT4621 showed that it acetylates specific amino acids (Section 5.1.1).

[4] Technical dossier/ Section D1

- *Zm-hra* is an optimised form of the endogenous acetolactate synthase (*als*) coding sequence from maize, which confers tolerance to ALS-inhibiting herbicides, such as chlorimuron, thifensulfuron and other sulfonylurea herbicides. Two point mutations were introduced within the *als* coding sequence, resulting in two amino acid changes (A165 and L542). As a result, the Zm-HRA protein provides tolerance to ALS-inhibiting herbicides.

Maize embryos of line PHWVZ were transformed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) strain LBA4404, using binary vector PHP24279, to produce genetically modified maize 98140. This vector PHP24279 harbours in its T-DNA region the *gat4621* and *Zm-hra* expression units in opposite orientations.[5]

- The *gat4621* coding sequence is under the regulation of the promoter of the maize ubiquitin gene (*ubi*1), including a 5′ untranslated region and an intron, and with transcription terminated by the proteinase inhibitor II (*pin*II) terminator from *Solanum tuberosum.*

- The *Zm-hra* coding sequence is under the regulation of the promoter of the endogenous *als* gene and the *pin*II terminator from *S. tuberosum.*

The expression of both genes, *gat4621* and Zm-*hra,* is enhanced by three copies of the CaMV 35S enhancer region from *Cauliflower mosaic virus* located in between the two genes.

Several functional sequences are located outside the T-DNA on the vector backbone, including, among, others *tet*R and *tet*A coding for tetracycline resistance and a gene coding for spectinomycin resistance.

3.1.2. Transgenic constructs in the genetically modified plant

Molecular analyses were undertaken on BC0S2, BC1 and T0S3 generations, produced after two generations of crossing the original transformation event 98140 with maize line PH09B.[6] Southern analysis, PCR, sequencing and inheritance studies established that a single, intact T-DNA was inserted into the maize nuclear genome to produce maize 98140. The absence of the whole vector backbone of PHP24279 plasmid in event 98140 has been confirmed by Southern blot analysis.[7]

The DNA sequence of the insert contains 7 386 base pairs. The nucleotide sequence of the flanking genomic regions were determined, extending 2 310 base pairs at the 5′ end and 2 027 base pairs at the 3′ end of the insert. Both flanking regions were shown to be maize genomic sequences. Bioinformatic analysis indicated that the inserted genetic modification has not interrupted any known maize coding sequences,[8] but it is inserted approximately 2.8 kb upstream of the *liguleless*-1 coding sequence involved in leaf morphology (Sylvester et al., 1990).

In order to assess whether the open reading frames (ORFs) present spanning the junction sites raise any safety issue, their putative translation products were compared with databases for similarities to known allergens and toxins using suitable algorithms.[9] No significant similarities were found.

3.1.3. Information on the expression of the insert

The expression levels of GAT4621 and Zm-HRA were measured by ELISA in several samples of maize 98140 cultivated in field trials at six locations during two seasons (2006 and 2007) in the United States and Canada.[10] Expression levels of GAT4621 and Zm-HRA were measured in plants that were either untreated or treated with combinations of herbicides (glyphosate, ALS-inhibiting herbicides or a

[5] Technical dossier/Section C.
[6] Technical dossier/Section D2.
[7] Additional information September 2009.
[8] Technical dossier/Section D2/Annex 4.
[9] Additional information March 2009/Annexes 1 and 2.
[10] Technical dossier/Section D3/Annex 6a and 6b.

combination of both). Levels of both proteins in plants untreated or treated with herbicides were in the same range. Below only data from untreated plants are summarised. In both 2006 and 2007, GAT4621 and Zm-HRA expression was measured in leaf, root, stalk, pollen, whole plant, forage and grain at four different growth stages. The mean expression of GAT4621 in grain was 7.9 ng/mg tissue dry weight (dw) (range 3.6–20.0 ng/mg dw) in 2006 and 8.8 ng/mg dw (range 3.6–14.0 ng/mg dw) in 2007. The mean expression of Zm-HRA in grain was 0.34 ng/mg tissue dw (range 0–0.92 ng/mg dw) in 2006 and 0.36 ng/mg tissue dw (range 0.27–0.69 ng/mg dw) in 2007. The potential impacts of the protein levels are assessed in the food/feed safety assessment sections.

3.1.4. Inheritance and stability of inserted DNA

Stability of the insert in maize 98140 was investigated by PCR and Southern blot analyses on individuals obtained from backcrosses and self-cross. The insert was stable and followed the Mendelian inheritance pattern of a single locus.[11] A further study across two generations confirmed the genetic and phenotypic stability of the insert.[12]

3.2. Conclusion

The molecular characterisation data provided by the applicant established that the genetically modified maize 98140 contains one copy of the T-DNA consisting of the *gat4621* and the *Zm-hra* cassettes. No other parts of the initial plasmid used to obtain the DNA fragment for transformation were detected in the transformed plant. Bioinformatic analysis of the 5′ and 3′ flanking regions did not reveal disruption of known genes or creation of ORFs that would cause a safety issue. The stability of the inserted DNA was confirmed over several generations and a Mendelian inheritance pattern was demonstrated. The EFSA GMO Panel concludes that the molecular characterisation does not raise safety issues.

4. Comparative analysis

4.1. Evaluation of the relevant scientific data

The GMO Panel has considered the data on the compositional, agronomic and phenotypic characteristics of maize 98140 and its comparators as provided in the dossier and summarised below.

4.1.1. Choice of comparator and production of material for the compositional assessment

A comparative analysis of the compositional, agronomic and phenotypic characteristics of maize 98140 and its comparator was performed during field trials at six locations in the United States and Canada in two subsequent years, 2006 and 2007.[13] The comparator used was a negative segregant that had been isolated, after several stages of backcrossing and selfing of the progeny of the initial transformant, from maize that was essentially homozygous for the insert in maize 98140. Each location contained four replicated blocks, each of which had four plots of maize 98140 treated with different herbicide regimes and one plot with the comparator. Three of the four blocks were designated for the production of samples for compositional analysis and for analysis of agronomic and phenotypic characteristics, while the remaining block was for expression analysis of the transgenic GAT4621 and Zm-HRA ALS proteins. The four herbicide treatment regimes of maize 98140 included three treatments with target herbicides (single or mixed applications of the intended herbicides: glyphosate; nicosulfuron and rimsulfuron; glyphosate, nicosulfuron and rimsulfuron) on top of maintenance herbicides and a treatment with maintenance herbicides only.

The design of the field trials performed in 2006 and 2007 was not considered by the EFSA GMO Panel as appropriate to demonstrate the absence of unintended effects because of limitations, namely the use of a negative segregant as the only comparator. As negative segregants are derived from a genetically modified organism, the GMO Panel does not consider them appropriate conventional counterparts with a history of safe use (EFSA, 2006a).

[11] Technical dossier/Section D3/Annex 3.
[12] Technical dossier/Section D3/Annex 2.
[13] Technical dossier/Section D7.2.

Following a request from the EFSA GMO Panel[14] for additional data incorporating an appropriate non-GM counterpart, in line with the EFSA (2006) guidance document, data on field trials performed in Europe in 2008 using a near-isogenic line as comparator were provided by the applicant. These data were obtained from field trials conducted at four locations in Spain and two locations in Romania. In these field trials maize 98140, in 09B/EHG background, was compared to the conventional counterpart maize PHWVZ, with a similar genetic background, obtained from a parallel breeding programme. Each location contained four replicated blocks, each of which had four plots of maize 98140 that were treated differently with regard to herbicide regimes and one plot with the conventional counterpart. Three out of the four maize 98140 blocks were designated for the production of samples for compositional analysis and for analysis of agronomic and phenotypic characteristics. The four different herbicide treatment regimes of maize 98140 included three different treatments with target herbicides (single or mixed applications of the intended herbicides: glyphosate; nicosulfuron and rimsulfuron; glyphosate, nicosulfuron and rimsulfuron) on top of maintenance herbicides and a treatment with maintenance herbicides only.

Data from field trials for the comparative compositional assessment are necessary to identify potential unintended effects. Requirements for the geographical spread of locations and the number of growing seasons need to meet minimum standards of representativeness of environments and statistical power (EFSA, 2006). This affords confidence that any unintended effect will be detected if it is present. The field trials performed in 2008 did not fulfil the requirement for multiple seasons in the applicable EFSA Guidance Document (EFSA, 2006), nor did they fulfil the requirements specified in the current (EFSA, 2011) guidance document. Because of this non-compliance with the requirements set out in its guidance, the EFSA GMO Panel deemed the data provided as insufficient to exclude the possibility of unintended effects.

4.2. Conclusion

The EFSA GMO Panel cannot conclude on the comparative assessment of the compositional, agronomic and phenotypic characteristics of maize 98140, on the basis of the data provided.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

In the absence of an appropriately performed comparative assessment, the Panel is not in a position to conclude on the compositional, agronomic and phenotypic characteristics of maize 98140 compared with conventional maize. The safety assessment could therefore not be completed, and has focused mainly on the newly expressed proteins and on specific metabolites resulting from the acetylase activity of the GAT4621 protein.

5.1.1. Non-specific acetylation[15]

Given its capacity to acetylate glyphosate the substrate specificity of the GAT4621 protein was explored *in vitro*, with regard to potential acetylation of other substrates using 21 amino acids, 10 antibiotics and 21 different agrochemicals. In incubation mixtures containing GAT4621 (produced in *E. coli*) and acetyl coenzyme A, acetylation was measured indirectly by the detection of coenzyme A release. In these studies GAT4621 acetylated aspartic acid, glutamic acid, serine, threonine and glycine with relatively low efficiency compared with the acetylation of glyphosate. The highest catalytic efficiency (k_{cat}/K_m) of the reaction with the amino acids, which was observed for aspartic acid and glutamic acid, was approximately 1 % of that for glyphosate. The affinity of the protein for glycine was so low that a K_m value could not be estimated. These findings are in line with the detection of elevated levels of several *N*-acetylated amino acids.[16] In particular *N*-acetyl-aspartic acid

[14] Additional information March 2010.
[15] Technical dossier/ Section 7.8.1/Annex 14.
[16] Technical dossier/Section D7.2.

(NAA) and *N*-acetyl-glutamic acid (NAG) were found in much higher concentrations in maize 98140 than that typically observed in conventional maize (Table 1).

Table 1: Mean levels of acetylated amino acids in maize 98140.[17]

	Year	Control maize	98140 *m*aize (mg/kg)
N-Acetylated amino acid			
N-acetyl-l-aspartic acid (NAA)	2008	1.01	542
	2007	4.03	292
	2006	0.90	403
N-acetyl-l-glutamate (NAG)	2008	0.05	13.0
	2007	0.21	84.0
	2006	0.50	79.0
N-acetylglycine (NAGly)	2008	0.06	0.12
	2007	0.06	0.12
	2006	0.07	0.23
N-acetylserine (NAS)	2008	1.16	2.03
	2007	0.86	1.49
	2006	0.90	1.97
Acetylthreonine (NAT)	2008	0.18	1.42
	2007	0.16	1.97
	2006	0.18	2.99

NAA and NAG were not detectable in refined bleached and deodorised oil. The levels of NAA and NAG in other processing fractions were less than those detected in the whole grain. In general, wet mill and dry grind (ethanol) processing reduced the concentrations of NAA and NAG relative to dry mill processing.[18]

5.1.2. Toxicology[19]

5.1.2.1. Proteins used for safety assessment

Given the low levels of expression of the proteins GAT4621 and Zm-HRA ALS in maize 98140 and the difficulty of producing sufficient amounts thereof for safety testing, recombinant proteins produced in the bacterium *Escherichia coli* were used for safety testing.

In the case of the Zm-HRA ALS protein, the mature form, which does not contain the chloroplast transit peptide that is cleaved from the protein during processing in the plant, was produced in *E. coli* in the form of a fusion protein. Owing to the cleavage of the fusion protein with thrombin during the purification process, the resulting microbial Zm-HRA ALS protein has three additional N-terminal

[17] Technical dossier/Section 7.1 and additional information March 2010.
[18] Additional information March 2010.
[19] Technical dossier/Section 7.8.

amino acid residues (glycine, serine and cysteine) compared with the protein expressed in maize 98140.

The functional equivalence of the bacterially produced GAT4621 and Zm-HRA ALS proteins to those expressed in maize 98140 was investigated and confirmed with the aid of various analytical techniques, including gel electrophoresis followed by protein staining, Western blot analysis, N-terminal sequencing and mass spectrometry (MALDI-MS of tryptic digests), and by the absence of protein glycosylation. The bacterially produced proteins were also assayed for molecular weight using electrospray mass spectrometry and enzyme activity.

Heat stability of the proteins

The stability of the GAT4621 and Zm-HRA ALS proteins, dissolved in buffer solutions at pH 7.2–7.5 and incubated at temperatures up to 60 °C for 15 minutes, was tested by measuring the residual activity of these enzymes after incubation compared with their activity in unheated solutions. GAT4621 lost more than 89 % of its activity at temperatures greater than 53 °C. Zm-HRA ALS completely lost its activity at 50 °C or higher.

5.1.2.2. Toxicological assessment of the newly expressed proteins in maize 98140[20]

The Zm-HRA ALS protein expressed in maize 98140 is an acetolactate synthase encoded by an optimised form of the endogenous *als* gene from Zea mays. ALS enzymes are key enzymes in the biosynthesis of the essential branched-chain amino acids, where they catalyse the first common step in the biosynthesis of isoleucine, leucine and valine, starting from pyruvate (LaRossa and Falco, 1984; Duggleby and Peng, 2000). The enzyme catalyses two reactions, these being the conversion of two molecules of pyruvate into 2-acetolactate, used in the synthesis of leucine and valine, and the condensation of pyruvate with 2-ketobutyrate producing 2-acetohydroxybutyrate, used in the synthesis of isoleucine. ALS enzymes are widespread in nature, and occur, for example, in plants, algae, yeast and bacteria (Falco and Dumas, 1985; Friden et al., 1985; Mazur et al., 1987; Mazur and Falco, 1989; Reith and Mulholland, 1995) and are therefore consumed as part of the human and animal diet. The Zm-HRA ALS protein expressed in maize 98140 is 638 amino acids in length and has a predicted molecular weight of 69 kDa for the full-length protein. As explained above, ALS proteins from plants contain an N-terminal chloroplast transit peptide that is removed after transport of the protein into the chloroplast. As a result, the length of the mature protein produced from both the endogenous ALS protein and the introduced Zm-HRA ALS protein is 598 amino acids, with a predicted molecular weight of 65 kDa. The amino acid sequence of the mature form differs from that of the endogenous maize protein in two amino acids (see Section 3.1.1). These changes confer tolerance to ALS-inhibiting herbicides to the modified maize.

The GAT4621 protein (147 amino acids, molecular mass *c*. 17 kDa) is an optimised form of the enzyme GAT from *B. licheniformis*, which acetylates glyphosate using acetyl coenzyme A as the acetyl donor. The coding sequence was obtained after 11 rounds of DNA shuffling using three distinct alleles of the *gat* gene isolated from three different strains of *B. licheniformis* (see Section 3.1.1). As no information is available on the safe consumption of this protein, further toxicological information has been requested by the EFSA GMO Panel.

(a) Bioinformatics studies

Based on the information obtained from the databases used, the analyses of the amino acid sequences of the newly expressed proteins GAT4621 and Zm-HRA revealed no significant similarities to known toxic proteins.

[20] Technical dossier/Section 7.8.1 and additional information March 2012.

(b) Resistance to degradation by proteolytic enzymes

The resistance to degradation by pepsin of each of the GAT4621 and Zm-HRA proteins was measured in solutions containing pepsin and the test protein at molar ratios of pepsin GAT4621 of 1.3:1 and of pepsin: Zm-HRA of 5.5:1, at pH 1.2. The integrity of the test protein in samples of the incubation mixture taken at various time points was analysed by gel electrophoresis followed by protein staining. It was thus observed that the full-length GAT4621 and Zm-HRA proteins were degraded within the first 30 seconds of incubation, while less intensely staining bands corresponding to low-molecular weight fragments were still visible on the electrophoresis gels near the dye front (≤ 3 kDa) throughout the incubation period. Also the resistance of both proteins to pancreatin at pH 7.5 was measured by protein staining and Western blotting with protein-specific antibodies after gel electrophoresis [pancreatin: test protein= 40:1 (v/v)]. It was thus observed that GAT4621 was degraded within five minutes of incubation, while Zm-HRA was degraded within 30 seconds.

(c) Acute toxicity testing

The proteins GAT4621 and Zm-HRA produced in *E. coli* did not induce adverse effects in separate acute oral toxicity studies using mice after administration of a single target dose of 2 g/kg body weight each (corresponding to actual doses of the proteins of at least 1.6 g/kg body weight (bw) of GAT4621 and approximately 1.2 g/kg bw of Zm-HRA).

The EFSA GMO Panel is of the opinion that acute toxicity testing of the newly expressed proteins is of little additional value for the risk assessment of the repeated consumption of food and feed from GM plants by humans and animals.

(d) Repeated-dose toxicity testing

Following a request from the EFSA GMO Panel to provide further information in order to demonstrate the safety of the GAT4621 protein, the applicant provided a repeated-dose toxicity study, in compliance with GLP requirements, using the GAT4621 protein produced in *E. coli* as test material. Groups of five male and five female mice (Crl:CD-1(ICR)) received standard rodent diets supplemented with the GAT4621 protein providing doses of 0, 81, 496 or 833 mg/kg bw per day (males) and 0, 97, 546 or 1034 mg/kg bw per day (females) for 28 consecutive days. Another control group received bovine serum albumin (BSA) at approximately 1 000 mg/kg bw per day.

Throughout the treatment period there were no deaths related to the test substance (the cause of the death of one male in the standard diet control group on day 27 was not identified). Regular observations of the animals revealed no clinically relevant findings that were considered to be related to the test material. Mean body weight gain and feed efficiency were statistically significantly higher during study days 14–21 in females of the high-dose group in relation to both control groups. Overall, body weight, body weight gain, feed consumption and feed efficiency were comparable in all groups. In ophthalmological examinations performed before the start of the treatment and at study days 26/27, no abnormalities were noted. In a functional observation battery (FOB) comprising detailed monitoring of the animals, sensory and neuromuscular measurements (in week 3) no treatment-related effects were identified.

Haematology and coagulation parameters were not analysed in this study, which is not in accordance with the recommendations in OECD Guideline 407 for the testing of chemicals (OECD, 2008). In clinical chemistry analyses performed at the end of the treatment period, males of all groups administered the GAT4621 protein showed higher total bilirubin blood levels compared with the standard diet control group (statistically significant in the low- and mid-dose groups). The change was not dose related and all values were similar to that of the BSA control group. High-dose females had significantly higher blood glucose levels compared with the standard diet control group, but the glucose levels were relatively high in all groups including the BSA control group. In the absence of changes in other parameters, these findings are not considered toxicologically relevant. The EFSA

GMO Panel notes, however, that the variation of many individual parameters was relatively wide. Organ weight determinations at necropsy showed significantly higher absolute and relative (in relation to final body weight and brain weight) testis weights in high-dose males compared with the standard diet control group. Testis weights were also higher in the BSA control group, and the microscopic examinations of this organ revealed no pathological findings. Therefore the higher testis weight is considered to be an incidental finding. Macroscopic and microscopic examinations of other selected organs and tissues revealed no gross or histopathological changes.

No adverse effects of the GAT4621 protein were detected. However the EFSA GMO Panel notes that the study was not performed in accordance with OECD Guideline 407 because relevant parameters, i.e. haematology and coagulation, were not analysed.

5.1.2.3. Toxicological assessment of changed levels in natural constituents[21]

The applicant provided published information on NAA, NAG, *N*-acetylserine (NAS), *N*-acetylthreonine (NAT) and *N*-acetylglycine (NAGly) and the full reports of the available toxicological studies. A summary of these studies has been published by Delaney *et al*. (2008), Harper *et al*. (2009 and 2010), Karaman *et al*. (2009) and van de Mortel *et al*. (2010a and 2010b).

Information on NAA and NAG

NAA and NAG are normal constituents of many foods, including maize (Hession et al., 2008) and are produced in mammalian metabolism. *N*-acetylation and de-acetylation of cellular proteins is a widespread process with a regulatory function in metabolism (Perrier *et al*, 2005; Smith and Denu, 2007; Hwang *et al*, 2010) and is mediated by a number of both specific and unspecific *N*-acetyltransferases that also acetylate free amino acids, amines and drugs. NAA is synthesised in brain neurons and has important roles in the function of the central nervous system. However, NAA must be released by neurons into the extracellular fluid from where it is taken up by glial cells to be hydrolysed to aspartic and acetic acid (Baslow, 2003, 2010). NAA of a different origin occurs also in other organs.

Ingested *N*-acetylated amino acids are presumably deacetylated by aminoacylase-1, the most abundant of the aminoacylases and expressed in all nucleated human cells, including the intestine and the kidneys, which means that under normal conditions dietary *N*-acetylated amino acids are not absorbed as such in the gut or excreted in the urine to a great extent (EFSA, 2003). Studies in mice using radiolabelled NAA and L-aspartic acid showed that after intraperitoneal injection both substances were metabolised at a similar rate (as determined by measurement of expired radioactive CO_2) indicating a rapid hydrolysis of the *N*-acetyl group.

Studies in premature infants, rats, dogs and pigs with enterally or parenterally administered *N*-acetylated amino acids (cysteine, tryptophan, tyrosine, methionine, threonine, glutamine) have shown that the nutritional value of the *N*-acetylated amino acids was comparable to that of free amino acids (Boggs, 1978; Neuhäuser-Berthold et al., 1988; Gouttebel et al., 1992; van Goudoever et al., 1994; Arnaud et al., 2004; López-Pedrosa et al., 2007).

Toxicological information

(a) Acute toxicity testing

In acute oral toxicity studies using male and female Sprague–Dawley rats there were no indications of adverse effects after administration of NAA, NAG, NAGly, NAS and NAT at doses of 2 000 mg/kg bw. When NAA was tested at a dose of 5 000 mg/kg bw four of five female animals in the test group died and the surviving female as well as all male rats showed signs of toxicity including ataxia, abnormal gait, noisy breathing or diarrhoea. The EFSA GMO Panel is of the opinion that acute

[21] Technical dossier/Section 7.8.2 and additional information July 2010.

toxicity testing is of limited additional value for the risk assessment of the repeated human and animal consumption of food and feed from GM plants.

(b) Repeated-dose toxicity testing[22]

28-day toxicity study with NAA

NAA was administered in the diet to groups of 10 male and 10 female Sprague–Dawley rats for 28 days. The study was conducted in compliance with GLP requirements and in accordance with OECD Guideline 407, apart from the selection of dose levels. During the first 14 days the animals received standard rodent diets to which the NAA was supplemented providing target doses of 0,10, 100 or 1 000 mg/kg bw per day and during the remaining period the target doses were 0, 100, 500 or 1 000 mg/kg bw per day. There were no deaths during the treatment period. Clinical signs as well as effects identified in ophthalmological examinations were not related to the test material. No relevant differences in body weight development, feed consumption and feed efficiency were found between the treatment groups and the control group. FOB and motor activity evaluations also did not reveal relevant differences. Urine analyses showed differences in ketone concentrations for male rats, which can be considered as normal variation. Haematology and clinical chemistry analyses showed several statistically significant differences in the high-dose group compared with the control group, i.e. a lower mean eosinophil count, lower levels of mean plasma creatinine and blood urea nitrogen and a higher plasma glucose level in males as well as a lower mean neutrophil count in females. These differences are not considered toxicologically relevant and most likely represent incidental findings. Other significant differences observed in only one of the lower dose groups and in one sex, are also regarded as incidental. Organ weight determinations as well as macroscopic and microscopic examination of organs and tissues at necropsy did not reveal relevant differences in findings between the test and control groups. Therefore, in this study no adverse effects were observed up to the highest dose administered. The no observed adverse effect level (NOAEL) in this study was the highest dose administered, which corresponds to an actual average dose of 852.3 mg/kg bw per day for males and 890.1 mg/kg bw per day for females.

28-day toxicity study with NAG

In a similar study Sprague–Dawley rats received standard rodent diets to which the NAG was supplemented providing target doses of 0, 100, 500 or 1 000 mg/kg bw per day for 28 days. An additional group received diet supplemented with L-glutamate at a target dose of 1 000 mg/kg bw per day for comparison. All animals survived during the treatment period and there were no relevant differences between groups regarding body weight development, feed intake and feed efficiency. FOB and motor activity evaluations as well as eye examinations did not reveal relevant findings. In haematology and clinical chemistry examinations, the only statistically significant differences in relation to the control group were a higher calcium level in females of the mid-dose group, which is considered incidental, as well as higher white blood cell and absolute lymphocyte counts in male rats of the high-dose group. Similar changes in blood cell counts were also observed in male rats receiving L-glutamate. In the absence of any other relevant findings in the other examinations (urinalysis, organ weight determinations, macroscopic and microscopic examinations), these differences, which were not observed in females, are most likely not attributable to administration of NAA (and L-glutamate, respectively). The NOAEL in this study was the highest dose administered, which corresponds to an actual average dose of NAG of 914.2 mg/kg bw per day for males and 1 006.6 mg/kg bw per day for females.

28-day toxicity study with NAS

Sprague–Dawley rats received standard rodent diets to which the NAS was supplemented providing target doses of 0, 100, 500 and 1 000 mg/kg bw per day for 28 days. An additional group received a

[22] Additional information December 2010.

diet with L-serine at a target dose of 1 000 mg/kg bw per day. One male animal in that group showed adverse clinical signs and had to be euthanised on day 11 of the treatment period. The cause (broken palate) was not considered related to the test material. There were no notable findings in clinical observations or ophthalmological examinations. FOB and motor activity evaluations did not reveal relevant differences between groups. Body weight, body weight gain, feed consumption and feed efficiency were comparable in all groups except for the high-dose group, which showed a higher (although not statistically significant) body weight and body weight gain due to a higher feed consumption. The only statistically significant difference in haematology and clinical chemistry analyses compared with the control group was a higher plasma alkaline phosphatase activity in females of the high-dose group. No difference was noted in males. In the absence of any other changes in related parameters, and considering that there were no relevant findings in the histopathological examinations (see below), this difference can be regarded as an incidental finding. Urinalysis results showed no remarkable changes. Organ weight determinations showed lower paired adrenal weight (absolute and in relation to brain weight but not in relation to body weight) in male animals of the mid-dose group as well as higher spleen weight in female animals of the low-dose group. These differences were unrelated to the dose and thus also considered incidental. Macroscopic examinations at necropsy, as well as microscopic examinations of selected organs and tissues, did not reveal toxicologically relevant effects. Therefore the highest dose administered, corresponding to an actual dose of NAS of 839.7 and 893.6 mg/kg bw per day for male and female animals, respectively, is regarded as the NOAEL in this study.

28-day toxicity study with NAT

Using the same study design, Sprague–Dawley rats received standard rodent diets to which NAT was supplemented providing target doses of 0, 100, 500 and 1 000 mg/kg bw per day over a period of 28 days. Also in this study, the unmodified amino acid L-threonine was administered to an additional group at a target dose of 1 000 mg/kg bw per day. All rats survived until scheduled sacrifice and treatment with NAT did not cause any adverse clinical signs. FOB and motor activity evaluations as well as ophthalmological examinations revealed no relevant differences between groups. Regarding body weight development, feed consumption, feed efficiency as well as haematology examinations, there were no statistically significant differences in test groups compared with the control group. In clinical chemistry parameters, males of the high-dose group showed a statistically significant lower serum aspartate aminotransferase (AST) activity in relation to the control group, a change in direction that is not regarded as an indication of toxicity. In females of the high-dose group, plasma sodium levels were significantly higher. In the absence of any changes in related parameters and taking account of the histopathological examinations (see below), these changes are not considered toxicologically relevant. Organ weight determinations showed a higher relative heart weight (in relation to brain weight) for male rats of the high-dose group. Higher absolute and relative heart weights (relative to body weight and brain weight) were also observed in the group administered L-threonine. Females of the high-dose group showed higher absolute and relative heart weights (in relation to body weight and brain weight). All mean values and most of the individual values were within the range of the historical control data. There were no changes in other parameters indicative of heart toxicity, and histopathological examinations of this organ revealed no adverse effects. Therefore the changes in heart weight are not considered toxicologically relevant. No relevant differences between groups were identified in the macroscopic and histopathological examinations of organs and tissues. Therefore the highest dose administered, corresponding to an actual dose of NAT of 848.5 and 913.6 mg/kg bw per day for male and female rats, respectively, is determined to be the NOAEL in this study.

28-day toxicity study with NAGly

Sprague–Dawley rats received standard rodent diets to which the NAGly was supplemented providing target doses of 0, 100, 500 or 1 000 mg/kg bw per day for 28 days. An additional group received glycine at a dose of 1 000 mg/kg bw per day. There were no deaths during the treatment period and no adverse clinical signs. Ophthalmological examinations revealed no remarkable effects. No relevant

differences in body weight development, feed consumption and feed efficiency were found between the treatment groups and the control group. Treatment did not affect any of the parameters evaluated in FOB and motor activity measurements. Urinalysis results were unremarkable. Analyses of haematology and coagulation parameters showed a statistically significantly prolonged activated partial thromboplastin time (APTT) for females of the mid-dose group compared with the control group, which is considered to be an incidental finding. Females of the high-dose group showed a prolonged prothrombin time (PT). All individual values fell within the range of the historical control data. Since the difference in relation to the control group was small (16.31 vs 15.48 seconds) this was not considered to be an adverse effect. Clinical chemistry analyses identified a lower serum albumin level in males of the mid-dose group, which was not dose related and therefore regarded as an incidental finding. Organ weight determinations showed a lower relative ovary weight (in relation to brain weight) in female rats of the high-dose group. As there were no differences in absolute weight and ovary weight in relation to body weight, and histopathological examinations of this organ revealed no changes, the difference in ovary weight relative to brain weight was considered incidental. Macroscopic and microscopic examination of organs and tissues at necropsy did not reveal relevant differences in findings between the test and control groups. Therefore the highest target dose administered in this study, corresponding to an actual dose of 898.9 and 989.9 mg/kg bw per day for male and female rats, respectively, is regarded as the NOAEL.

90-day toxicity study with NAA[23]

The applicant also provided a subchronic (90 days) feeding study with NAA using Sprague–Dawley rats. The study was conducted in accordance with OECD Guideline 408 and in compliance with GLP requirements. Groups of 10 male and 10 female animals received standard rodent diets to which NAG was supplemented providing target doses of 0, 100, 250 and 500 mg/kg bw per day. An additional group was administered L-aspartic acid at a target dose of 500 mg/kg bw per day. All animals survived the treatment period. Clinical observations as well as ophthalmological examinations did not reveal relevant differences between the treatment groups and the control group. FOB and motor activity evaluations also did not show relevant differences. Body weight, body weight gain, feed consumption and feed efficiency were comparable in all groups. Haematology examinations showed statistically significantly higher red blood cell counts in females of the high-dose group in relation to the control group. The difference was small and, in the absence of changes in related parameters, not considered toxicologically relevant. Other significant differences observed at lower dose levels were unrelated to the dose and thus regarded as incidental findings (higher white blood cell counts and lymphocyte counts in males; prolonged APTT in females). Clinical chemistry examinations showed lower blood urea nitrogen levels in males of the high-dose group, which is not considered as an indication of toxicity. This also applies to lower creatinine levels in males of the mid-dose group. Urine analyses showed no relevant differences. In organ weight determinations carried out at necropsy, males of the mid-dose group showed a higher relative heart weight (in relation to brain weight), which was not dose-related and not observed in relation to body weight and therefore regarded as incidental. A similar conclusion can be drawn for differences in thymus weights observed in female animals (thymus weight in relation to body weight was higher in the mid-dose group, whereas thymus weight in relation to brain weight was lower in the low-dose group). Females of all dose groups showed lower relative liver weights in relation to body weights, which was also not related to the dose level and, in the absence of other findings indicating liver toxicity, probably attributable to a relatively high value of the control group. Macroscopic examinations revealed numerous red areas of the thymus of a number of animals in all groups, in particular in female animals of the group administered L-aspartic acid, which was not further explained by the author of the study report but is not considered treatment related. Microscopic examinations did not reveal relevant differences between groups except for an increased incidence and severity of hypertrophy of the mucus-secreting cells (acinar cells) in the submandibular salivary gland of male and female rats of the high-dose NAA group (but not in the group administered 500 mg L-aspartic acid/kg bw per day). The cells were enlarged with an increased amount of pale, basophilic cytoplasm but there was no evidence of injury or cytotoxicity, e.g.

[23] Additional information December 2010.

inflammation, degeneration, necrosis or hyperplasia. This effect was observed at a lower incidence and intensity also in the parotid salivary glands (high-dose males and females) and in the sublingual salivary gland (high-dose males). The effect was not observed at a target dose of 250 mg NAA/kg bw per day. The EFSA GMO Panel concludes that the no observed effect level (NOEL) in this study is the mid-dose administered, corresponding to an actual average dose of 229.5 and 253.2 mg NAA/kg bw per day for male and female rats, respectively.

Reproductive and developmental toxicity study with NAA

NAA was tested in a two-generation reproduction toxicity study using rats, which was carried out according to OECD Guideline 416 and in compliance with GLP requirements. Rats received standard rodent diets to which the NAG was supplemented providing target doses of 0, 100, 250 and 500 mg/kg bw per day. An additional group was administered L-aspartic acid at a target dose of 500 mg/kg bw per day. Groups of 25 male and 25 female Sprague–Dawley rats (P_1 generation) continuously received the test diet starting 70 days before mating, through mating and continuing until sacrifice. F_1 generation rats received the same diet concentrations from weaning until sacrifice or for at least 70 days before mating, through mating and continuing until sacrifice. F_2 generation rats received the same diet concentrations from weaning until scheduled sacrifice. Several deaths occurring prior to scheduled sacrifice in the P_1, F_1 or F_2 generation were considered incidental. Regular observations of P_1, F_1 and F_2 animals did not reveal clinically relevant effects, and body weights, body weight changes, feed consumption and feed efficiency were comparable in all groups. Organ weight determinations and macroscopic and microscopic examinations at necropsy did not show relevant differences between groups except for hypertrophy of acinar cells of salivary glands in male and female rats in the F_1 generation and male rats from the F_2 generation of the high-dose NAA group. Neurohistopathological evaluation provided no evidence that NAA had any effects on brain development. Delivery or litter observations for the P_1 or F_1 generation females were not affected. There were also no signs of reproductive effects on the P_1 or F_1 generation males or females or effects on the viability and growth in the F_1 or F_2 generation offspring. Reproductive parameters evaluated in the F_1 and F_2 generation rats after weaning were not affected. The EFSA GMO Panel noted a decreased motor activity of male and female animals administered NAA or L-aspartic acid in one specific subset of the F_2 generation (examined on day 22-post partum). This was not observed in the F_1 generation, and in older animals of the F_2 generation (examined on day 61-post partum). Therefore the EFSA GMO Panel is of the opinion that the observed difference in motor activity is not attributable to NAA. The EFSA GMO Panel concludes that the NOEL in this study is the mid-dose administered, corresponding to an actual average dose of 245.7 and 269.1 mg NAA/kg bw per day for male and female rats for the F_1 generation, respectively and 237.2 for the males of the F_2 generation.

(c) Genotoxicity testing

Tests on induction of gene mutations in bacteria (Ames test) were conducted in accordance with OECD Guideline 471. Using the plate incorporation method NAA, NAG, NAGly, NAS and NAT did not induce gene mutations in *Salmonella* Typhimurium strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2*uvr*A up to the highest tested concentration of 5 000 µg/plate in the absence and presence of tissue homogenate with metabolic activity (S9-mix).

The acetylated amino acids were also tested in the mouse bone marrow micronucleus test which was conducted in accordance with OECD Guideline 474. Groups of 10 male and 10 female mice received by gavage NAA, NAG, NAGly, NAS and NAT at doses of 0, 500, 1 000 or 2 000 mg/kg bw or NAG at doses of 0, 333, 1 000 or 2 000 mg/kg bw. In these studies the test materials did not increase the frequency of micro nucleated polychromatic erythrocytes up to the highest dose level. However, there is no information on systemic uptake of the test substance which leads to uncertainty in the interpretation.

On the basis of the available information the EFSA GMO Panel concludes that there is no indication of genotoxicity.

5.1.2.4. Toxicological assessment of the whole GM food/feed[24]

(a) Repeated-dose (90-day) oral toxicity study

The applicant has provided the report on a repeated-dose (90-day) oral toxicity study with maize 98140 in rats (CRL:CD(SC)), which was also published in the scientific literature (Appenzeller et al., 2009). A total of six groups of 24 rats each, including 12 animals of each gender, received diets containing 35 38 % (w/w) milled grain from specific maize lines for approximately 90 days. The specific maize lines (collected from a single site in 2006 in the United States[25]) included maize 98140 treated with conventional herbicides, maize 98140 treated with glyphosate plus ALS inhibitors (nicosulfuron and rimsulfuron), a negative segregant (control maize designated 091), and three commercial non-GM maize varieties (33J56, 33P66 and 33R77). According to the study report, the diets contained comparable concentrations of proximate analytes, fibre, energy, amino acids, vitamins and minerals. The maize used for diet formulation was also checked for the presence of the GAT4621 and Zm-HRA ALS proteins.

All animals survived until termination of the experimental period, except for one male animal on a reference diet, for which the cause of death (due to pyelonephritis) was considered unrelated to the diet. While most of the time, no statistically significant differences in body weight, body weight gain, feed consumption and feed efficiency were observed between the test groups and the control group, female animals fed both diets containing maize 98140 showed a transient statistically significantly lower average body weight gain and average daily feed efficiency in one week of the experiment (day 7–14), and higher average daily feed intakes in several other weeks. However, there were no significant differences in average body weight and total body weight gain at the end of the treatment period.

In clinical chemistry analysis, male animals fed maize 98140 treated with conventional herbicides showed a statistically significantly higher average value of alkaline phosphatase activity compared with the control group, yet this difference was not observed in the group fed maize 98140 treated with glyphosate plus ALS inhibitors, and the average value fell within the range of the groups fed the three reference diets. In female animals fed maize 98140 treated with glyphosate plus ALS inhibitors, the alkaline phosphatase activity was significantly higher compared with the control, while no significant difference was observed in the group fed maize 98140 treated with conventional herbicides. In the absence of any changes in other parameters, these differences are regarded as incidental findings.

No effects of test diets, either statistically significant or otherwise apparent (except for the mortality mentioned above), were observed in the analysis of clinical observations, ophthalmology, FOB, motor activity, haematology, coagulation, urinalysis, organ weights, gross necropsy and histopathology.

The EFSA GMO Panel considers that a repeated-dose 90-day oral toxicity study, in which material derived from a negative segregant is administered as the sole control material, has limitations for the safety assessment of food/feed from GM plants. In the present study three commercial reference varieties were included. There were no indications of adverse effects after administration of grain from maize 98140 in relation to the control group receiving grain from a negative segregant, and the values and/or nature of findings in these groups were within the ranges determined for the three groups receiving grain from different commercial reference varieties.

(b) Broiler growth study

A 42-day feeding study using broiler chickens was provided.[26] A total of 720 Ross × Cobb broiler chicks at hatch were subdivided into six groups, each group consisting of 120 broilers housed in 10 pens (five males and five females per pen). The six groups received diets containing grains from maize

[24] Technical dossier/Section 7.8.4 and additional information July 2012.
[25] Technical dossier/Section 7.6.
[26] Technical dossier/ Section D.7.8.4/Annex 22.

98140 grown under conventional agriculture practices (test group), maize 98140 sprayed with intended herbicide regime (test group), a negative segregant (control group), or three commercial non-GM maize varieties (reference group: 33J56, 33P66, 33R77). The maize 98140 was confirmed by the presence of transgenic DNA by PCR and newly expressed proteins by ELISA.

Before mixing the feed all maize grains were analysed for proximates, amino acids, fatty acids and mycotoxins. Diets were formulated to meet the minimum nutrient requirements of a typical commercial broiler diet (NRC, 1994). At three stages during the diet preparation (beginning, middle and end), the contents of NAA and NAG in each individual diet were also determined.[27]

Each group of chickens was fed consecutively with starter, grower, and finisher diets containing 58.5 %, 64 % or 71.5 % of maize grain respectively. Chickens were observed three times daily for signs of adverse effects; any deaths were recorded and a full necropsy conducted. Body weight and feed intake were measured every seven days.

A statistically significantly increased liver weight was observed in females fed the two diets containing maize 98140 (averages 3.51–3.71 % of relative live bird weight) compared with animals of the control group (average 3.34 %), while the range in animals fed commercial diets ranged between 3.50 % and 3.62 % (McNaughton et al., 2008). No other statistically significant changes in average values were noted in the comparison between chicken fed diets containing transgenic maize and the control group. Moreover, this change in liver weight was not observed in female chicken in a similar broiler chicken feed study in which animals received diets containing a combination of 98140 maize grain and meal, hulls and oil of soybean 356043, the latter containing a similar genetic modification as maize 98140, with newly expressed GAT and ALS enzymes (McNaugthon et al., 2011b).

The EFSA GMO Panel considers that the use of a negative segregant as the sole control material has limitations for the safety assessment of food/feed from GM plants. In this study three commercial non-GM maize varieties were included. Both feed intake and growth rate did not differ significantly between animals fed the test materials, the negative segregant and the non-GM varieties. The feeds used in the study were nutritionally satisfactory and there was no indication of adverse effects on the performance of the animals fed diets containing maize 98140 compared with the diets containing the negative segregant or conventional maize. These outcomes are in line with the absence of adverse effects in other published studies in which diets containing a combination of maize 98140 and soybean 356043 were fed to broiler chickens and laying hens (McNaughton et al., 2011a,b).

5.1.3. Allergenicity[28]

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food.

5.1.3.1. Assessment of allergenicity of the newly expressed proteins

A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (CAC, 2009; EFSA, 2011).

The source of the transgene encoding the GAT4621 protein, i.e. *Bacillus licheniformis*, is not known to be allergenic, as well as that of the Zm-HRA protein, i.e. maize, is not considered to be allergenic.

Based on the information obtained from the databases used, analyses of the amino acid sequences of the newly expressed proteins GAT4621 and Zm-HRA revealed no significant similarities to known allergens.

[27] Technical dossier/ Section 7.6/Table 21.
[28] Technical dossier/Section 7.9.

As summarised in Section 5.1.2.2b) the GAT4621 and Zm-HRA proteins were degraded in solutions containing the proteases pepsin and pancreatin.

Based on the available information and considering the EFSA guidance document (2006a), the EFSA GMO Panel considers that there are no indications that the newly expressed GAT4621 and Zm-HRA proteins in maize 98140 may be allergenic in the intended conditions of exposure.

5.1.3.2. Assessment of allergenicity of the whole GM plant or crop

Since no reliable information is available from the comparative analysis and in light of its relevance for the identification of possible unintended effects, the EFSA GMO Panel cannot conclude on the allergenicity of the whole GM plant.

5.1.4. Nutritional assessment of GM food/feed

5.1.4.1. Intake information/exposure assessment

NAA and NAG are present in conventional foodstuffs and feeds, and are thus normal constituents of the human and animal diet (see Section 5.1.2.3). The applicant provided analytical data using HPLC/mass spectrometry for a range of foodstuffs, which were selected because they have relatively high concentrations of aspartic acid and glutamic acid. NAA and NAG were detectable in whole egg, dried egg powder, chicken bouillon, ground beef and ground turkey (Table 2).[29] Using UPLC tandem quadruple mass spectrometry, a more sensitive method, the applicant showed that NAA and NAG are also present in other foodstuffs including canned sardines, apples, oranges, spinach, brown rice, barley flour, wheat grain, walnuts, barley beer, green coffee beans, dried tea, cocoa powder and chocolate.[30]

On request of the EFSA GMO Panel the applicant provided a dietary exposure assessment for NAA and NAG by substituting conventional maize with maize 98140.[31] Separate calculations were carried out using data provided by the US Dietary Exposure Evaluation Model—Food Commodity Intake Database (DEEM-FCID) and the Global Environment Monitoring System—Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diets, respectively.

However, the dietary exposure assessments for NAA and NAG, provided by the applicant, were predominantly on the basis of consumption data obtained from the US population; secondly, the NAA and NAG contents in maize 98140 were measured in grains harvested at one single site of the field trial in 2006, whereas considerable variation was seen for NAA and NAG contents in grains harvested from other field trials.[32] Consequently EFSA performed an additional dietary exposure assessment using the EFSA Comprehensive Food Consumption Database (EFSA, 2011) as the data source.

As the intake of NAA and NAG from conventional maize would be negligibly small (see Table 1), the contribution of conventional maize was not included. The intake scenario applied refers to a total replacement of all conventional maize in food by the 98140 maize.

[29] Technical dossier/Section 7.3/Annex 11.
[30] Additional information March 2010/Annex 10.
[31] Additional information March 2010.
[32] Technical dossier/Section 7.3/Annex 9.

Table 2: Content of NAA and NAG in foods other than maize

Food description[1]	Foodex code and food group[2]	NAA[1] (mg/kg fresh weight)	NAG[1] (mg/kg fresh weight)
Ground beef	A.01.000729 Beef meat (*Bos* spp.)	1.07	1.53
	A.01.000730 Veal meat	1.07	1.53
Ground turkey	A.01.000738 Turkey meat (*Meleagris gallopavo*)	3.98	0.79
Chicken boullion	A.01.000737 Chicken meat (*Gallus domesticus*)	12.11	0.36
Dried egg powder	A.01.001263 Eggs, powder	6.94	0.70
Whole egg	A.01.001253 Egg, fresh	1.38	0.05

1 Data provided by the applicant (see Technical dossier/Tables 18 and 19).
2 Reference numbers in this column are from the EFSA comprehensive database.

The average concentration of NAA and NAG (514 and 16.9 µg/g dw, respectively), was measured in grains harvested from six sites in the 2008 field trial, in which maize 98140 was treated with intended herbicides (glyphosate, nicosulfuron and rimsulfuron). A moisture content of 10 % was assumed for the maize grain. As the effects of processing could not be established quantitatively (see Section 5.1.1), no correction factor was introduced for processed foods. The intake levels of NAA or NAG by consuming other foods (chicken meat, turkey meat, beef meat, fresh egg and dried egg powder) were also calculated (see Table 2). The concentration of NAA or NAG in these foods was determined on raw uncooked products.[33] The EFSA comprehensive database was also used to construct the intake levels in various age classes (Table 3) in the EU population. The levels shown include the variability between different national surveys by Member States, through calculation of summary statistics from the various surveys submitted by Member States for each age class. For example, the 95th percentile for each of the 12 surveys for the adolescent age class was calculated, and the quoted estimate of 513 µg/kg bw per day in the column labelled 'Maximum' represents the largest of these 12 values. As shown in Table 3, the intake of NAA was generally two orders of magnitude higher than that of NAG. The intake in any age class was in the magnitude of micrograms (µg) per kg of body weight per day with two exceptions which were about 1 mg/kg bw per day.

Table 3: Estimated total daily dietary intake of NAA and NAG in the EU population (including 100 % replacement of maize products with the GM maize 98140)

NAA	Mean (µg/kg bw per day)			95th percentile (µg/kg bw per day)			
Age class	*N*_surveys*	Minimum	Median	Maximum	*N*_surveys*	Minimum	Median	Maximum
Infants	2	2	25	49	1	120	120	120
Toddlers	7	25	38	297	4	72	608	1069
Other children	15	25	81	264	15	105	428	994
Adolescents	12	24	50	169	12	32	276	513
Adults	15	11	27	71	15	22	128	291

33 Technical dossier/Section 7.3/Annex 11.

NAA	Mean (µg/kg bw per day)				95th percentile (µg/kg bw per day)			
Age class	*N*_surveys*	Minimum	Median	Maximum	*N*_surveys*	Minimum	Median	Maximum
Elderly	7	6	12	27	7	12	44	204
Very elderly	6	7	9	24	5	22	25	181

NAG	Mean (µg/kg bw/day)				95th percentile (µg/kg bw day)			
Age class	*N*_surveys*	Minimum	Median	Maximum	*N*_surveys*	Minimum	Median	Maximum
Infants	2	0.4	1.2	2.0	1.0	8.7	8.7	8.7
Toddlers	7	1.4	1.7	12	4.0	6.5	24	39
Other children	15	1.3	3.5	10	15	5.3	14	33
Adolescents	12	0.8	2.8	5.7	12	2.0	10	17
Adults	15	0.4	1.4	3.2	15	1.1	5.5	10
Elderly	7	0.4	0.9	1.8	7.0	1.5	2.8	8.0
Very elderly	6	0.4	1.0	1.6	5.0	1.7	2.7	6.5

*Only if the number of participants in a survey exceeds 60 were dietary data collected from that survey used to calculate the 95th percentile.

The EFSA GMO Panel has no safety concerns for humans regarding the elevated levels of NAA in maize 98140. The calculated margin of 250–2 500 between the estimated amounts of NAA consumed in a worst-case scenario, including 100 % replacement of conventional maize by GM maize at the 95th percentile in all age groups (*c*. 0.1–1.0 mg/kg bw per day) and the highest dose administered without effects in the 90-day toxicity study (*c*. 250 mg/kg bw per day; see Section 5.1.3.4a), is considered sufficient. Moreover, the estimated intake of NAA is small in comparison with the habitual dietary intake of aspartic acid (7.3 g aspartic acid plus asparagine per day; HCN, 1999). Similarly for NAG, the Panel concludes that there are no safety concerns, considering the anticipated intake levels, the outcome of the 28-day repeated dose oral toxicity study (see Section 5.1.3.3) and the habitual intake of glutamic acid (8.5 g per day; HCN, 1999).

5.2. Conclusion

In the absence of an appropriately performed comparative assessment, the EFSA GMO Panel is not in the position to conclude on the compositional, agronomic and phenotypic characteristics of maize 98140, on the basis of the data provided. The safety assessment could therefore not be completed, and has focused mainly on the newly expressed proteins and on specific metabolites resulting from the acetylase activity of the GAT4621 protein.

No safety concerns were identified for the newly expressed protein ZM-HRA, based on the widespread occurrence of ALS enzymes in nature and as part of the human and animal diet, lack of known toxicity to humans or animals and lack of similarity to known toxins in bioinformatics analyses. The GAT4621 protein did not show significant similarity to known toxins in bioinformatics analyses either. Based on the available information and considering the EFSA guidance document (2006a), the EFSA GMO Panel considers that there are no indications that the newly expressed GAT4621 and ZM-HRA, proteins in maize 98140 may be allergenic in the intended conditions of exposure.

A repeated-dose (28-day) oral toxicity study using mice with GAT4621 was not performed in accordance with the respective OECD Guideline because relevant parameters (i.e. haematology and coagulation) were not analysed. Therefore, the EFSA GMO Panel cannot conclude on the safety of the newly expressed GAT4621 protein.

Feeding studies in laboratory rats and broiler chickens did not show adverse effects of administering diets containing maize 98140 to these animals compared with diets containing a negative segregant of maize 98140 or conventional maize.

The calculated margins between the estimated intakes of NAA and NAG, and the highest doses administered without observed effects in the available toxicological studies in animals, are considered sufficient. Moreover, the estimated intakes of NAA and NAG are small in comparison with the habitual dietary intakes of aspartic acid and glutamic acid, respectively. The EFSA GMO Panel is of the opinion that the higher levels of NAA and NAG as such do not raise safety concerns for humans or animals.

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

The scope of this application, EFSA-GMO-UK-2008-53, is for food and feed uses and import and processing of maize 98140 and does not include cultivation. Considering the intended uses of maize 98140, the environmental risk assessment is concerned with exposure through manure and faeces from animals fed grains (F_2 generation) from maize 98140 and with the accidental release into the environment of viable grains of maize 98140 during transport and processing.

Maize 98140 has been developed to be tolerant to the herbicide glyphosate and to ALS-inhibiting herbicides, such as chlorimuron, thifensulfuron and other sulfonylureas. Herbicide tolerance is achieved by the introduction of the *gat4621* and the Zm-*hra* coding sequences surrounded by their necessary regulatory components (see Section 3.1.2).

6.1.1. Environmental risk assessment

6.1.1.1. Potential unintended effects on plant fitness due to the genetic modification

Maize is highly domesticated and generally unable to survive in the environment without management intervention. Maize plants are not winter hardy in many regions of Europe; furthermore, they have lost their ability to release seeds from the cob and they do not occur outside cultivated land or disturbed habitats in agricultural landscapes in Europe, despite cultivation for many years. In cultivation, maize volunteers may arise under some environmental conditions (mild winters). Observations made on cobs, cob fragments or isolated grains shed in the field during harvesting indicate that grains may survive and overwinter in some regions, resulting in volunteers in subsequent crops. The occurrence of maize volunteers has been reported in Spain and other European regions (e.g. Gruber et al., 2008). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs *et al*., 2009).

The applicant presented agronomic and phenotypic data gathered over a series of field trials conducted in the United States and Canada in 2006 and 2007[34]. These field trials were not accepted by the EFSA GMO Panel due to limitations in the study design, namely the use of a negative segregant as the only comparator. Upon request of the EFSA GMO Panel, the applicant provided additional data[35] on agronomic and phenotypic characteristics of the GM maize from field studies in 2008. However the field trials performed in 2008 were conducted in an insufficient number of seasons and locations (one year, six locations: for further details, see Section 4) compared with the requirements laid down in the EFSA Guidance document (EFSA, 2006).

[34] Technical dossier/Section D7.2.
[35] Additional information March 2010.

The EFSA GMO Panel is not aware of any other scientific reports on the spread and establishment of maize 98140 or of any change in survival capacity, including overwintering.

Therefore, given the limitations of the field trials provided by the applicant to support its risk assessment of maize 98140, the EFSA GMO Panel has identified a gap in the data on the agronomic and phenotypic characterisation of this event and considers that there remains uncertainty over these characteristics. However, the EFSA GMO Panel concludes that, considering the scope of this application, the available data and the poor ability of maize to survive outside cultivated land, there is very little likelihood of environmental effects due to the accidental release into the environment of viable grains from maize 98140.

6.1.1.2. Potential for gene transfer[36]

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either horizontal gene transfer of DNA or vertical gene transfer via seed spillage followed by cross-pollination.

(*a*) *Plant to bacteria gene transfer*

Genomic plant DNA is a component of several food and feed products derived from maize. It is well documented that DNA present in food and feed becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to bacteria in the digestive tract of humans, domesticated animals and other environments exposed to the GM plant or plant material is expected.

Current scientific knowledge of recombination processes in bacteria suggests that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (see EFSA, 2009, for further details).

A successful horizontal transfer would require stable insertion of the transgene sequences into a bacterial genome and a selective advantage to be conferred on the transformed host. The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is homologous recombination. This requires the presence of stretches of DNA sequences that are similar in the recombining DNA molecules and, in addition to substitutive gene replacement, facilitates the insertion of non-homologous DNA sequences if their flanking regions share sequence similarity with bacterial sequences in the recipient.

Maize event 98140 was developed through *Agrobacterium tumefaciens*-mediated transformation and contains genetic elements of which one has high similarity to a bacterial gene. The introduced genes are the *gat4621* gene, optimised for increased GAT catalytic activity in plants, originating from *B. licheniformis*, and an optimised form of the *zm-hra* gene originating from *Zea mays*, for increased tolerance to ALS[37]-inhibiting herbicides. The *Zm-hra* gene is of plant origin but with minor nucleotide modifications in the coding region and altered combinations of plant regulator sequences. Homology of this gene to genomic sequences of environmental bacteria that would facilitate homologous recombination is not expected. Acetolactate synthase genes have been detected in various bacteria and therefore recombination with homologous DNA sequences (substitutive recombination) and expression of the *Zm-hra* gene into bacterial genomes, which is not expected to occur, would not add new properties to recipient organisms.

B. licheniformis, the species from which the gat4621 was derived, is a spore-forming soil bacterium which is not considered to be prevalent in the gastrointestinal tract of humans or animals fed maize

[36] Technical dossier/Section D6
[37] ALS = acetolactate synthase.

98140. Due to the generally wide environmental spread of bacterial spores, it can however not be excluded that occasionally spores of *B. licheniformis* may also occur in these main receiving environments. Furthermore, outside this main route of exposure through food and feed use, recombinant DNA of maize 98140 may also accidentally come into contact with *B. licheniformis* cells in soil. Therefore, various routes of exposure have been considered here.

On a theoretical basis (i.e. in the absence of experimental evidence of horizontal gene transfer in GM food and feed derived from maize 98140 or any other GM plant), it can be assumed that, as an extremely rare event, homologous recombination may occur in the environment between the recombinant *gat4621* gene and their natural variants as they may occur in *B. licheniformis*. Such recombination events would only replace such natural variants (substitutive recombination), and therefore a new property conferring a selective advantage for the recipient organisms is not expected (EFSA, 2009).

The *gat4621* coding sequence is under the regulation of the promoter of the maize ubiquitin gene (*ubi*1) and with transcription terminated by the proteinase inhibitor II (*pin*II) terminator from *S. tuberosum.* The Zm-*hra* coding sequence is under the regulation of the promoter of the endogenous *als* gene and the *pin*II terminator from *S. tuberosum* (see Section 3.1.2). The expression of eukaryotic promoters in bacteria is generally inefficient (Warren et al., 2008).

In addition to homology-based recombination processes, illegitimate recombination that does not require similarity between the recombining DNA molecules is theoretically possible. However, transformation rates for illegitimate recombination are considered to be 10^{10}-fold lower than for homologous recombination (Hülter and Wackernagel, 2008; EFSA, 2009). Illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM-plant DNA (EFSA, 2009). Thus, this process, compared with homologous recombination, is considered not to contribute significantly to horizontal gene transfer events. In comparison with the above-described homology-facilitated recombination processes, the contribution of illegitimate recombination is extremely low.

Owing to the bacterial origin of the *gat4621* gene and the prevalence of bacterial genes encoding for acetolactate synthase in the environment, a low-level gene transfer to *B. licheniformis* or other bacterial species is thought not to confer a new trait and selective advantage. Considering its intended use as food and feed and the above assessment, the EFSA GMO Panel has therefore not identified a concern associated with a potential horizontal gene transfer from maize 98140 to bacteria.

(b) Plant to plant gene transfer

Considering the intended uses of maize 98140 and the physical characteristics of maize seeds, possible pathways of gene dispersal are grain spillage and the dispersal of pollen from occasional feral GM maize plants originating from accidental grain spillage during transport and/or processing.

The extent of cross-pollination to other maize varieties will mainly depend on the scale of accidental release during transportation and processing and on successful establishment and subsequent flowering of this GM maize plant. For maize, any vertical gene transfer is limited to other Zea mays plants as populations of sexually compatible wild relatives of maize are not known in Europe (Eastham and Sweet, 2002; OECD, 2003).

The flowering of occasional feral GM maize plants originating from accidental release during transportation and processing is unlikely to disperse significant amounts of GM maize pollen to other maize plants. Field observations performed on maize volunteers after GM maize cultivation in Spain revealed that maize volunteers had a low vigour, rarely had cobs and produced pollen that cross-pollinated neighbour plants only at low levels (Palaudelmàs et al., 2009).

Although GM maize plants outside cropped areas have been reported in Korea, as a result of grain spillage during import, transport, storage, handling and processing (Kim et al., 2006; Lee et al., 2009; Park et al., 2010), survival of maize plants outside cultivated fields in Europe is mainly limited by a combination of low competitiveness, the absence of a dormancy phase and susceptibility to plant pathogens, herbivores and frost. As for any other maize varieties, GM maize plants would only survive in subsequent seasons in warmer regions of Europe and are not likely to establish feral populations under European environmental conditions.

The EFSA GMO Panel takes into account the fact that this application does not include cultivation of maize 98140 within the EU so that the likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered to be extremely low. In conclusion, considering the scope of this application, the available data and the poor ability of maize to survive outside cultivated land, the EFSA GMO Panel is of the opinion that there is very little likelihood of environmental effects as a consequence of spread of genes from this GM maize in Europe.

6.1.1.3. Potential interactions of the GM plant with target organisms

Considering the intended uses of maize 98140, excluding cultivation and the absence of target organisms, potential interactions of the GM plant with target organisms were not considered an issue.

6.1.1.4. Potential interactions of the GM plant with non-target organisms

Owing to the intended uses of maize 98140, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered an issue by the EFSA GMO Panel.

6.1.1.5. Potential interaction with the abiotic environment and biogeochemical cycles

Owing to the intended uses of maize 98140, which exclude cultivation, and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

6.2. Post-market environmental monitoring

The objectives of a monitoring plan, according to Annex VII of Directive 2001/18/EC, are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the environmental risk assessment.

Monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the monitoring plan provided by the applicant (EFSA, 2011b). The potential exposure to the environment of maize 98140 would be through manure and faeces from animals fed maize 98140 grains and/or through accidental release into the environment of GM maize grains (e.g. during transportation and processing). The scope of the monitoring plan provided by the applicant is in line with the intended uses. As the environmental risk assessment did not identify potential adverse environmental effects due to maize 98140, no case-specific monitoring is required.

The general surveillance plan proposed by the applicant includes (1) the description of an approach involving operators (federations involved in maize import and processing) reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of the information recorded by the various operators; and (3) the use of networks of existing surveillance systems (Lecoq *et al*., 2007; Windels *et al*., 2008). The applicant proposes to submit a general surveillance report on an annual basis.

The EFSA GMO Panel is of the opinion that the scope of the monitoring plan proposed by the applicant is in line with the intended uses of maize 98140, as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the monitoring plan.

6.3. Conclusion

Considering the intended uses of maize 98140, the environmental risk assessment is concerned with indirect exposure, mainly through manure and faeces from animals fed grains from maize 98140, and with the accidental release into the environment of viable maize 98140 grains (e.g. during transportation and processing). The EFSA GMO Panel has identified a gap in the data on the agronomic and phenotypic characterisation of GM maize 98140 and considers that there remains uncertainty over these characteristics. However, considering the scope of this application, the available data and the poor ability of maize to survive outside cultivated land, the EFSA GMO Panel concludes that there is very little likelihood of environmental effects due to the accidental release into the environment of viable grains from maize 98140. Considering its intended uses as food and feed, interactions with the biotic and abiotic environment were not considered to be an issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from maize 98140 to bacteria have not been identified. The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of maize 98140 and the guidance document of the EFSA GMO Panel on post-market environmental monitoring of GM plants (EFSA, 2011). The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the monitoring plan.

CONCLUSIONS AND RECOMMENDATIONS

The molecular characterisation data establish that the genetically modified maize 98140 contains a single insert consisting of the *gat4621* and the *Zm-hra* expression cassettes. Bioinformatic analyses and genetic stability studies did not raise safety issues. The levels of the GAT and HRA protein in maize 98140 have been sufficiently analysed.

The EFSA GMO Panel considered the compositional, agronomic and phenotypic data supplied in the light of the field trial design. In this application the minimum standards for the design of field trials set out in the EFSA GMO Panel Guidance Document, were not met and therefore the EFSA GMO Panel cannot conclude on the comparative assessment of compositional, agronomic and phenotypic characteristics, on the basis of the data provided. In the absence of conclusions on the comparative assessment of composition, an assessment was restricted to the newly expressed proteins and to specific metabolites resulting from the acetylase activity of the GAT4621 protein. No safety concerns were identified for the newly expressed protein Zm-HRA ALS, based on the widespread occurrence of ALS enzymes in nature and as part of the human and animal diet, the lack of known toxicity to humans or animals and lack of similarity to known toxins in bioinformatics analyses. The GAT4621 protein did not show significant similarity to known toxins in bioinformatics analyses either. However, the repeated-dose (28-day) oral toxicity study provided using mice fed with GAT4621 was not performed in accordance with the respective OECD Guideline because relevant parameters (i.e. haematology and coagulation) were not analysed. Therefore, the EFSA GMO Panel cannot conclude on the safety of the newly expressed GAT4621 protein. The elevated levels of the natural constituents NAA and NAG in maize 98140 were also assessed. The EFSA GMO Panel is of the opinion that the higher levels of NAA and NAG as such would not raise safety concerns for humans or animals.

Considering the intended uses of maize 98140, which exclude cultivation, there is no requirement for a scientific assessment of possible environmental effects associated with the cultivation of this GM maize. The EFSA GMO Panel has identified a gap in the data on the agronomic and phenotypic characterisation of GM maize 98140 and considers that uncertainty over these characteristics remains. However, considering the scope of this application, the available data and the poor ability of maize to survive outside cultivated land, the EFSA GMO Panel concluded that there is very little likelihood of environmental effects due to the accidental release into the environment of viable grains from maize

98140. Considering its intended use as food and feed, interactions with the biotic and abiotic environment were not considered to be an issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from maize 98140 to bacteria have not been identified. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize 98140 and the guidance document of the EFSA GMO Panel on post-market environmental monitoring of GM plants (EFSA, 2011b). In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of maize 98140. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the monitoring plan.

In the absence of an appropriately performed comparative assessment by the applicant, the EFSA GMO Panel was not in a position to complete its risk assessment on maize 98140 and therefore does not conclude on the safety of maize 98140 compared with its conventional counterpart with respect to potential effects on human and animal health. However, the EFSA GMO Panel concludes that the maize event 98140 is unlikely to have any adverse effect on the environment in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the United Kingdom, received on 15 April 2008, concerning a request for the placing on the market of genetically modified maize 98140 submitted under Regulation (EC) No 1829/2003 by Pioneer.

2. Acknowledgement letter, dated 23 April 2008, from EFSA to the Competent Authority of the United Kingdom.

3. Letter from EFSA to applicant, dated 10 June 2008, requesting additional information under completeness check.

4. Letter from applicant to EFSA, received on 8 October 2008, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 12 November 2008, delivering the "Statement of Validity" for application EFSA-GMO-UK-2008-53, regarding genetically modified maize 98140 submitted under Regulation (EC) No 1829/2003 by Pioneer.

6. Letter from EFSA to applicant, dated 23 January 2009, requesting additional information and stopping the clock.

7. Letter from applicant to EFSA, received on 17 March 2009, providing additional information.

8. Letter from EFSA to applicant, dated 20 May 2009, requesting additional information and maintaining the clock stopped.

9. Letter from applicant to EFSA, received on 6 October 2009, providing additional information.

10. Letter from applicant to EFSA, received on 5 March 2010, providing additional information.

11. Letter from EFSA to applicant, dated 28 May 2010, requesting additional information and maintaining the clock stopped.

12. Letter from applicant to EFSA, received on 12 July 2010, providing additional information.

13. Letter from EFSA to applicant, dated 26 October 2010, requesting additional information and maintaining the clock stopped.

14. Letter from applicant to EFSA, received on 22 December 2010, providing additional information.

15. Letter from EFSA to applicant, dated 10 February 2011, requesting additional information and maintaining the clock stopped.

16. Letter from applicant to EFSA, received on 8 March 2012, providing additional information.

17. Letter from EFSA to applicant, dated 24 May 2012, requesting additional information and maintaining the clock stopped.

18. Letter from applicant to EFSA, received on 13 July 2012, providing additional information.

19. Letter from EFSA to applicant, dated 30 November 2012, re-starting the clock.

20. Acknowledgement letter, dated 23 April 2008, from EFSA to the Competent Authority of the United Kingdom (Ref. PB/KL/shv (2008) 2969194).

21. Letter from EFSA to applicant, dated 10 June 2008, with request for clarifications under completeness check (Ref. SM/shv (2008) 3080244).

22. Letter from applicant, dated 6 October 2008, providing EFSA with an updated version of the application EFSA-GMO-UK-2008-53 submitted by Pioneer Overseas Corporation under Regulation (EC) No 1829/2003.

23. Letter from EFSA to applicant, dated 12 November 2008, delivering the "Statement of Validity" for application EFSA-GMO-UK-2008-53, maize 98140 submitted by Pioneer Overseas Corporation under Regulation (EC) No 1829/2003 (Ref. PB/SM/shv (2008) 3450887).

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