SCIENTIFIC OPINION

Scientific opinion on applications EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46 for the renewal of authorisation of maize T25,[1] and for the placing on the market of herbicide-tolerant genetically modified maize T25,[2] both for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience AG

EFSA Panel on Genetically Modified Organisms (GMO)[3,4]

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ABSTRACT

This scientific opinion reports on a risk assessment for the authorisation for (continued) marketing of genetically modified maize T25 for food and feed uses, import and processing. Maize T25 contains a single insertion locus containing a *pat* cassette conferring tolerance to glufosinate-based herbicides. Bioinformatic analyses, protein expression data and genetic stability studies did not raise safety issues. The compositional, agronomic and phenotypic characteristics of maize T25 grain and its conventional counterpart showed no differences that are of relevance for food/feed safety. The safety assessment identified no concerns regarding the potential toxicity and allergenicity of the newly introduced PAT protein. The compositional data indicating the nutritional equivalence of maize T25 were supported by the results of the feeding studies. There was no evidence that the genetic modification might significantly change the overall allergenicity of maize T25. Considering all available information related to the agronomic and phenotypic characterisation of maize T25, the EFSA GMO Panel did not observe any enhanced fitness characteristics of maize T25 that will change its capacity to spread, establish or persist compared with non-genetically modified (GM) maize, except in the presence of glufosinate-based herbicides. Considering its intended uses as food and feed, interactions with the biotic and abiotic environment were not considered an issue. Risks associated with an unlikely but theoretically possible horizontal gene transfer from maize T25 to bacteria have not been identified. The monitoring plan and reporting intervals were in line with the intended uses. The Panel concluded that maize T25, as described in the applications, is as safe as its conventional counterpart with respect to potential effects on human and animal health or the environment in the context of its intended uses for food and feed, import and processing.

[1] On request from the European Commission for an application (EFSA-GMO-RX-T25) submitted by Bayer CropScience AG, Question No EFSA-Q-2007-155, adopted on 11 September 2013.

[2] On request from the Competent Authority of the Netherlands for an application (EFSA-GMO-NL-2007-46) submitted by Bayer CropScience AG, Question No EFSA-Q-2007-134, adopted on 11 September 2013.

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KEY WORDS

GMO, maize T25, food and feed safety, environment, import and processing, Regulation (EC) No 1829/2003, herbicide tolerance

SUMMARY

Following a request from the Competent Authority of the Netherlands and from the European Commission (EC), the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on applications EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46, both submitted by Bayer CropScience AG under Regulation (EC) No 1829/2003.[5]

Application EFSA-GMO-RX-T25 was initially for renewal of the authorisation for continued marketing of:

- foods and food ingredients produced from maize T25 which have been placed on the market in accordance with Art. 5 of Regulation (EC) No 258/97;[6]
- feed containing, consisting of or produced from maize T25 (feed materials and feed additives) which has been placed on the market in accordance with Part C of Directive 90/220/EEC;[7]
- seeds for cultivation; Commission Decision of 22 April 1998 concerning the placing on the market of genetically modified maize (*Zea mays* L. T25), pursuant to Council Directive 90/220/EEC.

After the date of entry into force of Regulation (EC) 1829/2003, the products mentioned above were notified to the EC in accordance with Article 8(1)(b) or 20(1)(b) of this Regulation and subsequently included in the Community Register of genetically modified (GM) food and feed.

Application EFSA-GMO-NL-2007-46 was initially for food and feed uses, import and processing of maize T25 and all derived products, and cultivation of maize T25 in the European Union (EU).

Following the applicant's request to modify the scope of applications EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46 to no longer include cultivation of maize T25 in the EU, the EFSA GMO Panel provides a single scientific opinion, valid for both applications.

The EFSA GMO Panel assessed maize T25 with reference to the intended uses and appropriate principles described in its Guidance Documents for the risk assessment of GM plants and derived food and feed (EFSA, 2006a) and for renewal of authorisations of existing GMO products lawfully placed on the market (EFSA, 2006b). In delivering its scientific opinion, the EFSA GMO Panel considered applications EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46, additional information submitted by the applicant at the request of the Panel, the scientific comments submitted by Member States and relevant scientific publications. In accordance with its guidance document for renewal of authorisations of existing GMO products (EFSA, 2006b), the EFSA GMO Panel took into account the new information, experience and data on maize T25 that became available during the authorisation period. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of the target proteins. Evaluation of the comparative analysis of agronomic and phenotypic traits and composition was undertaken and the safety of the new proteins and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment was made of environmental impacts and the necessity for a post-market environmental monitoring plan.

[5] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 1–23.
[6] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 1–6.
[7] Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. OJ L 117, 15–27.

The molecular characterisation data established that the genetically modified maize T25 contains a single insertion locus containing a *pat* cassette. Bioinformatic analyses, protein expression data and genetic stability studies did not raise safety issues.

The EFSA GMO Panel concluded that the compositional, agronomic and phenotypic characteristics of grain maize T25 and its conventional counterpart showed no differences of relevance for food/feed safety.

The EFSA GMO Panel has evaluated the safety of the PAT protein in the context of the present application and several previous applications, and no safety concerns were identified.

The EFSA GMO Panel considered that there are no indications of concern relevant for food and feed safety arising from the comparative compositional assessment of maize T25. The compositional data indicating the nutritional equivalence of maize T25 were supported by the results of feeding studies with dairy cows and chickens. In addition, there is no evidence that the genetic modification might significantly change the overall allergenicity of maize T25. The EFSA GMO Panel concluded that maize T25 is as safe and nutritious as its conventional counterpart in the context of its intended use.

Applications EFSA-GMO-RX-T25 and EFSA-GMO-UK-2007-46 concern food and feed uses, import and processing. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of maize T25. In accordance with its guidance document on the environmental risk assessment of GM plants (EFSA GMO Panel, 2010), the EFSA GMO Panel follows a weight of evidence approach in collating and assessing appropriate information from various data sources (e.g. molecular and compositional data, available agronomic and phenotypic data from field trials performed by the applicant, literature) in order to assess the likelihood of unintended effects on the environment. Considering all available information related to the agronomic and phenotypic characterisation of maize T25, the EFSA GMO Panel did not observe any enhanced fitness characteristics of maize T25 that will change its capacity to spread, establish or persist compared with non-GM maize, except in the presence of glufosinate-based herbicides. 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 T25 to bacteria have not been identified. The scope of the post-market environmental monitoring plan provided by the applicant was in line with the intended uses of maize T25. Furthermore, the EFSA GMO Panel agreed with the reporting intervals proposed by the applicant in the post-market environmental monitoring plan.

In conclusion, the EFSA GMO Panel considered that maize T25, as described in applications EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46, is as safe as its conventional counterpart with respect to potential effects on human and animal health or the environment in the context of its intended uses for food and feed, import and processing.

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BACKGROUND

On 24 April 2007, the European Food Safety Authority (EFSA) received from the Netherlands Competent Authority an application (EFSA-GMO-NL-2007-46) for authorisation of genetically modified (GM) maize T25 (Unique Identifier ACS-ZMØØ3-2) submitted by Bayer CropScience AG within the framework of Regulation (EC) No 1829/2003 on GM food and feed. After receiving the application EFSA-GMO-NL-2007-46, and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission (EC) and made the summary of the application publicly available on the EFSA website.[8] 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 10 June 2008, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

On 29 June 2007, EFSA received from the EC an application (EFSA-GMO-RX-T25) submitted under Regulation (EC) No 1829/2003 for renewal of the authorisation of maize T25 for all uses with the exception of food and food products derived from maize T25.

The scope of the renewal application, as described in the Community Register,[9] covers the continued marketing of:

- Foods and food ingredients produced from maize T25 and all the varieties derived from starch and all its derivatives, crude, refined oil and all heat-processed or fermented products obtained from hominys, grits and flour (dry milled fragments) notified as existing foods falling within the scope of Article 8(1)(a) of Regulation (EC) No 1829/2003, which is produced from a genetically modified organism (GMO) and which have been placed on the market in accordance with Art. 5 of Regulation (EC) No 258/97;

- Feed containing, consisting of, or produced from maize T25 (feed materials and feed additives) notified as existing feed falling within the scope of Article 20(1)(a) of Regulation (EC) No 1829/2003, which consists of and/or contains a genetically modified organism (GMO) which has been placed on the market in accordance with Part C to the Directive 90/220/EEC;

- Seeds for cultivation; Commission Decision of 22 April 1998 concerning the placing on the market of genetically modified maize (*Zea mays* L. T25), pursuant to Council Directive 90/220/EEC.

After receiving the renewal application EFSA-GMO-RX-T25 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed Member States as well as the EC and made the summary of this application publicly available on the EFSA website.[10] EFSA initiated a formal review of the renewal application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 9 October 2008, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

On 26 February 2008, following a call for expression of interest among Competent Authorities under Directive 2001/18/EC and in accordance with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003, EFSA requested the United Kingdom Competent Authority (UK CA) to evaluate the initial environmental risk assessment of application EFSA-GMO-NL-2007-46 for the placing on the market of maize T25 for cultivation.

EFSA made the valid applications EFSA-GMO-NL-2007-46 and EFSA-GMO-RX-T25 available to Member States and the EC, and consulted nominated risk assessment bodies of Member States,

[8] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-134
[9] http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=20
[10] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-155

including national Competent Authorities within the meaning of Directive 2001/18/EC,[11] to request their scientific opinion. The Member State bodies had 3 months after the date of receipt of the valid application (until 10 September 2008 and 9 January 2009, respectively) within which to make their opinion known.

The UK CA asked the applicant for additional information on maize T25 on 1 October 2008. The applicant provided the requested information on 1 July 2009.

The UK CA provided to EFSA its report on the environmental risk assessment of maize T25 on 17 February 2012 in line with Articles 6.3(c) and 18.3(c) of Regulation (EC) No 1829/2003.

On 14 January 2013, EFSA received from the applicant a request to modify the scope of applications EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46 to no longer include cultivation of maize T25 in the EU.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out an evaluation of the risk assessment of the applications on maize T25 in accordance with the new scope and appropriate principles described in its guidance documents for the risk assessment of GM plants and derived food and feed (EFSA, 2006a) and for renewal of authorisations of existing GMO products lawfully placed on the market (EFSA, 2006b). In addition, the scientific comments of Member States, the additional information provided by the applicant and relevant scientific publications were taken into consideration.

For both EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46, the EFSA GMO Panel requested additional information from the applicant on 29 March 2012 and 24 May 2012. The applicant provided information related to both requests on 3 September 2012 and 18 December 2012. Following the change of scope in January 2013, the GMO Panel requested a PMEM plan according to the new scope on 4 February 2013. The applicant provided the requested information on 19 February 2013.

On 11 July 2012, EFSA informed the applicant that, given that applications EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46 had been processed for five years since their reception and the datasets received did not allow EFSA to conclude on the safety of maize T25, after 1 September 2012, which corresponded to the latest deadline to deliver additional information as specified by the applicant, EFSA would proceed with the finalisation of the assessment of EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46 and deliver its opinion based on the information available at that time.

In giving its scientific opinion on maize T25 to the EC, 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 6 months from the acknowledgement 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, this scientific opinion 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 respective overall opinions in accordance with Articles 6(5) and 18(5).

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific assessment of maize T25 (Unique Identifier: ACS-ZMØØ3-2) in the context of applications EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46. Considering the change of scope requested by the applicant, the scope of EFSA-GMO-RX-T25 covers the renewal of authorisation of (1) foods and food ingredients produced from maize T25 which have been placed on the market in accordance with Art. 5 of Regulation (EC) No 258/97 and (2) feed containing, consisting of, or produced from maize T25 (feed materials and feed additives)

[11] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L106, 1–39.

which has been placed on the market in accordance with Part C to the Directive 90/220/EEC, and application EFSA-GMO-NL-2007-46 is for food and feed uses, import and processing of maize T25 and all derived products.

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 a scientific opinion on information required under Annex II of the Cartagena Protocol. Furthermore, the EFSA GMO Panel did not consider 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 risk management.

ASSESSMENT

1. Introduction

Maize T25 was developed to express the enzyme phosphinothricin acetyl-transferase (PAT), encoded by the *pat* gene from *Streptomyces viridochromogenes* codon-optimised for expression in plants. Expression of PAT confers tolerance to glufosinate-based herbicides.

The genetic modification in maize T25 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, the processing characteristics and the overall use of maize as a crop.

The GM maize T25 (Unique Identifier: ACS-ZMØØ3-2) was evaluated with reference to its intended uses, taking account of the appropriate principles described in the Guidance Documents of the EFSA GMO Panel for the risk assessment of GM plants and derived food and feed (EFSA, 2006a) and for the renewal of authorisations of existing GMO products lawfully placed on the market (EFSA, 2006b).

The scope of application EFSA-GMO-RX-T25 covers the renewal of authorisation of (1) foods and food ingredients produced from maize T25 which have been placed on the market in accordance with Art. 5 of Regulation (EC) No 258/97 and (2) feed containing, consisting of or produced from maize T25 (feed materials and feed additives) which has been placed on the market in accordance with Part C of Directive 90/220/EEC, and the scope of EFSA-GMO-NL-2007-46 is for food and feed uses, import and processing of maize T25 and all derived products, as for any other commercial maize variety.

The risk assessment presented here is based on the information provided in applications EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46 submitted in the EU including the additional information from the applicant and the scientific comments that were raised by Member States on these applications.

A review of peer-reviewed scientific data on maize T25 and derived food and feed available since the original authorisation was provided by the applicant and did not raise issues impacting on the safety of maize T25 for humans and animals and for the environment.

The applicant also provided a report on the areas and quantity of production, importation and utilisation of maize T25 in Europe and information on known and estimated human and animal exposure.

A report on any unintended and/or unanticipated effects of maize T25 was not deemed applicable by the applicant as no general surveillance or case-specific monitoring was specified in the authorisation decision, and not provided.

Sections 2 to 7 report on the risk assessment for application EFSA-GMO-RX-T25 and Section 8 provides an assessment for the extended scope as indicated in EFSA-GMO-NL-2007-46.

2. Issues raised by the Member States

The scientific issues raised by the Member States are addressed in Annex G of the EFSA overall opinion and have been considered in this scientific opinion.[12,13]

[12] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-134
[13] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-155

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs

Maize T25 was developed by polyethylene glycol (PEG)-mediated transformation of protoplasts from the embryogenic cell suspension cultures of the maize genotype HE/89. The plasmid used for transformation contained a synthetic *pat* gene codon-optimised for expression in plants under the control of 35S promoter and terminator sequences derived from the *Cauliflower mosaic virus* (CaMV). The plasmid also included a β-lactamase (*bla*) gene and a bacterial origin of replication from pUC18.[14]

3.1.2. Transgene constructs in the genetically modified plant[15]

Data from Southern analysis (updated in 2012 with material produced in 2006 and 2012)[16] demonstrated that T25 maize contains a single insertion. The sequences actually inserted were determined by PCR amplification and sequence analysis. The insert contains a single copy of the P35S-*pat*-T35S expression cassette. The cassette is flanked at the 5′ end by 604 bp of the pUC18 plasmid including 5 bp of the *bla* gene and a fragment the *lacZ* gene. At the 3′ end, the cassette is flanked by 1 840 bp of the pUC18 plasmid including the origin of replication and a 665-bp 3′ fragment of the *bla* gene. The remainder of the *bla* gene (about 25 %) is not present in the insert.

Analysis of the flanking sequences of the insert confirmed that these were maize sequences. Bioinformatic analyses (updated in 2012)[17] showed that both the 3′ and 5′ flanking sequences have a high degree of similarity to the *Huck-2* retrotransposon, present at high copy number in the maize genome. Considering that the majority of the maize genome is derived from transposable elements, insertion in a retrotransposable element is not unexpected and does not raise a safety concern. These analyses also showed that no known endogenous genes were disrupted by the insertion.

An updated bioinformatic analysis (2012)[18] was performed on all possible open reading frames (ORFs) within the insert and spanning the maize genomic DNA–T25 insert junctions. None of the theoretical peptides deduced from these new ORFs showed relevant similarity with known toxins or allergens.

3.1.3. Information on the expression of the insert[19]

Analysis of PAT protein expression was carried out by ELISA using plants grown in four different field locations in Canada in 1995, in the USA in 1995 and 2009 and in France in 2004.[20] Considering the scope of the application, PAT protein levels in grain are considered the most relevant. Expression in grain ranged from below the limit of quantification to 0.43 μg/g dry weight.[21] Variations in protein expression values are not unexpected and can be due, for example, to differences in genetic background of the plants and/or environmental variables. Considering the nature of the PAT protein and the scope of the application, these variations in protein expression do not raise safety issues.

Although a complete *bla* gene is not present in transformation event T25, β-lactamase assays and Northern blot analysis were performed and no partial *bla* transcripts or β-lactamase activity were detected.

[14] Technical dossier/Section C.
[15] Technical dossier/Section D2.
[16] Additional information August 2012/Verhaeghe (2012b).
[17] Additional information August 2012/Verhaeghe (2012a).
[18] Additional information August 2012/Rascle (2012).
[19] Technical dossier/Section D3.
[20] Additional information August 2012.
[21] Additional information December 2012.

3.1.4. Inheritance and stability of inserted DNA

The inheritance pattern of the T25 event was investigated by Southern analysis of plant material obtained from the original transformant and from individuals of the third back-crossing generation. The probe corresponding to the *pat* gene produced the expected band in all cases, indicating stability of the integrated DNA. Updated Southern analyses of a later T25 generation grown in 2012[16] confirmed the stability of the integrated DNA. The phenotypic stability was determined following the segregation of the trait over two generations after being introgressed into different inbred lines. Stability was also confirmed by evaluating the segregation of the tolerance to glufosinate-ammonium in crosses between hemizygous T25 and non-GM inbreds. The inheritance pattern of the glufosinate tolerance trait was consistent with a single genetic locus segregating in a Mendelian fashion.

In conclusion, the stability of the inserted DNA and associated trait was confirmed over several generations.

3.2. Conclusion

The molecular characterisation data provided by the applicant established that the genetically modified maize T25 contains a single insertion locus containing a *pat* cassette. Updated bioinformatic analyses of the insert and flanking regions and protein expression data did not raise safety issues. The stability of the inserted DNA and the herbicide tolerance trait were confirmed over several generations and a Mendelian inheritance pattern was demonstrated. The EFSA GMO Panel concluded that the molecular characterisation does not raise safety issues.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

Compositional data were previously provided and assessed by the European Commission's Scientific Committee on Plants in its opinion published in 1998, in the frame for the approval of the environmental release including animal feed use of T25 maize under Directive 90/220/EEC (SCP, 1998), and by the UK Advisory Committee on Novel Foods and Processes (ACNFP) in the frame of a notification for human food use of processed products derived from T25 maize as novel food under Regulation (EC) No 258/97 (ACNFP, 1997). The data provided with the dossier for the current application were obtained after the publication of these opinions. As the ACNFP had requested monitoring of compositional data over time, the applicant provided new compositional data in 2002 (from 15 locations in the seasons 1999 and 2000, concurring with some of the data provided to EFSA; see Section 4.1.1). The ACNFP thus concluded that it was satisfied that the majority of components measured in T25 maize kernels fell within standard reference ranges for maize and that any of the statistically significant differences found between the three different test groups (GM maize treated or non-treated with target herbicide, conventional counterpart) were not of biological significance when viewed in the context of normal ranges (ACNFP, 2003).

In the present application, the applicant provided data on sweet maize grown in the USA during two seasons, as well as compositional data on field maize. The sugar profiles of the sweet maize kernels indicated that compositional changes during post-harvest storage could not be excluded. The EFSA GMO Panel therefore decided not to consider these data. Thus, the compositional characteristics of maize T25 were based on data from field maize only.

No data on forage composition were provided.

4.1.1. Choice of comparator and production of material for the comparative analysis

The field trials for the comparative compositional analysis of maize T25 and its conventional counterpart were carried out in 3 locations in France in 1999[22] and in 12 European locations in 2000, 4

[22] Technical dossier /Oberdoerfer (2002a).

each in Germany, France and Spain.[23] A randomised complete block design with four replications in 1999 and three replications in 2000 was used. The treatments included plots with T25 maize treated with a glufosinate-ammonium-containing herbicide, T25 maize treated with maintenance pesticides, and the conventional counterpart treated with the same maintenance pesticides. In the 1999 field trials, the T25 event was tested in two different maize genetic backgrounds, i.e. in Cecilia [LL (Liberty Link®) Moldova] and Torino (LL Kingston). The pedigree of the comparators indicated a genetic background very similar to those of the two maize T25 varieties. In the field trials in 2000, event T25 was tested in Torino background (LL Kingston) in six locations and in Anjou 400 background (LL Anjou 400) in six locations.[24] For additional analysis of the vitamin B6 content, the applicant performed an additional field trial in one location in the USA in 2006.[25]

The four field trials performed in France in 2000 were used for studying the agronomic and phenotypic characteristics of maize T25 (treated and untreated with the target herbicide) and its conventional counterpart (Anjou 400 in two locations and Torino in two other locations) as well as for harvesting material for the compositional analysis.[26] Additional agronomic information obtained from field trials performed in 1995 in Canada was also submitted by the applicant.

An additional field trial for the agronomic and phenotypic characterisation was conducted in the 2011/2012 growing season at two locations in São Desidério, Bahia, and Porto Nacional, Tocantins, in Brazil. Maize T25 and its conventional counterpart (the non-GM inbreed isoline B73) were used in a randomised complete block design with four replications. Maize T25 was used untreated (hand-weeded) and treated with the intended herbicide (glufosinate-ammonium; 3.0 L/ha)[27].

In order to evaluate the levels of the various constituents analysed in maize T25 and its conventional counterpart, the applicant provided data from the literature on the ranges in the level of these constituents in maize kernels and derived products.

4.1.2. Compositional analysis[28]

Grain of field maize grown in Europe during the seasons of 1999 and 2000 was analysed for proximates and dietary fibre, as well as for free and total amino acids, free and total fatty acids, vitamins (B1, B2, B6[29], niacin, pantothenic acid, folic acid, tocopherols), and for the anti-nutrient phytic acid. The selection of compounds concurred with the recommendations developed later by OECD (2002) except that various secondary analytes (raffinose, ferulic acid, p-coumaric acid, furfural, and trypsin inhibitor) have not been included.

The outcome of the compositional analysis on field maize grain of T25 and its conventional counterparts grown in Europe in 1999 and 2000 indicated that the level of the majority of maize constituents were comparable, but that there were statistically significant differences across locations in copper, total palmitic acid and total linolenic acid. However, the differences were not observed at a majority of the locations when the data were analysed by location. The levels of these analytes fell within the background ranges obtained from the literature.

The EFSA GMO Panel considered the observed compositional differences between grain produced from maize T25 and from its conventional counterpart in the light of the field trial design, measured biological variation and the level of the studied compounds in non-GM conventional varieties. The Panel concluded that no biologically relevant differences were identified in the compositional characteristics of grain produced from maize T25 compared with its conventional counterpart, and that

[23] Technical dossier/Oberdoerfer (2002c, 2006b).
[24] Additional information September 2012.
[25] Technical dossier/Oberdoerfer (2007).
[26] Technical dossier/Oberdoerfer (2004b, 2006b).
[27] Additional information June 2013.
[28] Technical dossier/Section D7.1.
[29] Field trial performed in USA, 2006.

its composition falls within the range of non-GM conventional varieties, except for the expression of the PAT protein.

4.1.3. Agronomic traits and GM phenotype[30]

The applicant presented agronomic and phenotypic data gathered from field trials with maize T25 across two locations in Canada in 1995, four locations in France in 2000 and two locations in Brazil over the 2011–2012 growing season. The EFSA GMO Panel considered the field trials carried out in Canada in 1995 as not appropriate since neither a conventional counterpart nor the intended herbicide treatment of maize T25 were included. The GMO Panel considered that the French and Brazilian field trials were in accordance with the applicable guidance document (EFSA, 2006a). However, the Panel noted that only five endpoints (plant count, plant height, diameter ears/average diameter of the spikes, male flowering/time to anthesis and spike height/length of ears) were measured in both of these trials.[31]

Of these five endpoints, only 'time to anthesis' (across locations and in two locations) in France showed statistically significant differences in maize T25 compared with its conventional counterpart, while no statistically significant differences were observed in Brazil in 2011–2012.

The EFSA GMO Panel examined other, publicly available agronomic and phenotypic data on maize T25 performance in variety trials which included a non-GM comparator. Maize variety Chardon LL containing the T25 event is reported to be similar to its conventional counterpart Orient (except for the glufosinate resistance trait). Maize variety Orient showed a field trial performance comparable to other non-GM maize varieties, as indicated in the national variety register of The Netherlands[32]. These reported summary data were insufficient for a full assessment by the EFSA GMO Panel owing to the limited availability of information on the study design and the statistical treatment of the data.

Evaluating all the available data and evidence on agronomic and phenotypic characteristics of maize T25 and its comparators, the EFSA GMO Panel is of the opinion that no indication of unintended effects that might raise safety concerns was observed.

4.2. Conclusion

The EFSA GMO Panel concluded that the compositional, agronomic and phenotypic characteristics of maize T25 grain and its conventional counterpart showed no differences of relevance for food/feed safety.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Effect of processing[33]

Maize T25 will be used for production and manufacturing of food and feed products in the same way as any other commercial maize variety.

[30] Technical dossier/Section D7.4.

[31] Parameters measured: Canada 1995: plant count, moisture, yield, stalk and root lodging, plant disease; France 2000: time of anthesis, plant height, plant count, yield, length and diameter of ears; Brazil 2011–2012: shape of the first leaf tip, angle between leaf blade and stem, behaviour of the leaf blade above the upper spike, length of the main tassel stem, angle between the main tassel stem and the side branching, colour of the stigma by anthocyanin, male and female flowering, plant height, spike height, final stand, average length of the spike, average diameter of the spikes, number of kernels per row, spike stuffing degree, spike compaction, weight of 1 000 seeds, type of kernel.

[32] Dutch variety registration data on Chardon LL and its non-GM counterpart Orient: "75e Rassenlijst voor Landbouwgewassen 2000" [75th List of Varieties of Field Crops 2000], Commissie voor de Samenstelling van de Rassenlijst voor Landbouwgewassen [Commission for the Compilation of the Variety List of Field Crops], p/a Centrum voor Plantenveredelings en Reproduktieonderzoek (CPRO) [c/o Center for Plant Breeding and Reproduction Research (CPRO)], Wageningen, the Netherlands, 2000, pp. 133–147.

[33] Technical dossier/Section D7.6.

The levels of the PAT protein in wet and dry milled field maize fractions as well as in processed sweet maize commodities were determined. For dry milling, the grains used for preparing the different matrices presented a PAT protein level of 130 ng/g. The PAT protein levels in the matrices were approximately 46 ng/g in hulls, 45 ng/g in grits, 17.2 ng/g in combined meal, 12.9 ng/g in flour and 276 ng/g in germ. In crude and refined oil from maize T25 grains, the PAT protein was not detectable. For wet milling, the grains used for preparing the different matrices presented a PAT protein level of 69.3 ng/g. The PAT protein levels in the matrices were approximately 7.7 ng/g in pressing cake from germs, 8.6 ng/g in meal after extraction of germs and 8.3 ng/g in meal after toasting germs.

Taking into account the compositional analysis, providing no indication of biologically relevant compositional changes except for the PAT protein, the EFSA GMO Panel has no reason to assume that the characteristics of maize T25 and derived processed products would be different from those of the respective products derived from conventional maize varieties except for the presence of the newly expressed protein.

5.1.2. Toxicology[34]

5.1.2.1. Toxicological assessment of the newly expressed protein

The EFSA GMO Panel has evaluated the safety of the PAT protein in the context of several previous applications and no concerns were identified (EFSA, 2005, 2006c, 2007a, b, c, 2008; EFSA GMO Panel, 2011c, 2012).

In the current application (EFSA-GMO-RX-T25), an updated bioinformatic analysis of the amino acid sequence of the PAT protein was provided. No significant similarities to known toxic proteins were found.[35]

5.1.2.2. Toxicological assessment of new constituents other than proteins

No new constituents other than the PAT protein have been deliberately introduced into maize T25 and no biologically relevant changes in the composition of grain from maize T25 were detected (see Section 4.1.2).

5.1.2.3. Animal studies with the food/feed derived from maize T25

a) Sub-chronic toxicity study

In a sub-chronic, 13-week rodent-feeding study, groups of 10 male and 10 female rats (strain Wistar Rj:WI (IOPS HAN)) received diets containing maize T25 milled maize at 33 % (w/w) and 11 % (w/w) inclusion levels (the former supplemented with 22 % B73 maize), the conventional counterpart B73 (inclusion level 33 %) or the commercial reference Hybrid 8223 (inclusion level 33 %).

Animals were housed in cages with five rats of the same sex per cage, but the data analysis considered the individual animal as the experimental unit, ignoring a possible bias due to cage interaction. Since the cage should be considered the experimental unit and because of the low number of experimental units per treatment (two per sex) a statistical analysis of the data is not possible. Therefore, the Panel did not consider this study in its evaluation.

b) Chicken feeding study

Reports of two 42-day feeding studies with broiler chickens were provided. In the first,[36] a total of 280 Ross × Ross male broiler chicks at hatch were subdivided into two groups, each group consisting of 140 broilers housed in four pens. The two groups received diets containing grain from maize T25 (test group) or an unspecified non-GM commercial maize variety. Diets were formulated to meet the

[34] Technical dossier/Section D7.8.
[35] Technical dossier/Section D7.8.1 and additional information September 2012.
[36] Technical dossier/Section D7.8.4/ Leeson (1996).

minimum nutrient requirements of a typical commercial broiler diet (NRC, 1994), and confirmed by the composition analysis of all diets. Each group of chickens was fed consecutively with starter, grower and finisher diets containing approximately 57 %, 61 % and 66 % of maize grain respectively.

However, the EFSA GMO Panel was unable to consider the results from this study given the lack of a conventional counterpart as the control material for feed formulation, the low number of experimental units and the application of parametric statistical analysis.

In the second 42-day feeding study,[37] a total of 420 one-day-old chicken (Ross 708, half male and half female) were randomly assigned to one of the three treatments, each treatment consisting of 14 replicate pens (seven pens per gender, 10 birds per pen). The diets, containing approximately 40 % maize grain, were formulated to meet poultry nutrient requirements and to be isocaloric and isonitrogenous, and balanced for limiting amino acids (analytically confirmed). Diets with the grains from the test diet (maize T25) contained the *pat* gene, but the control and the reference (non-GM commercial variety) diets did not.

Effects on health, survival, live weight, total weight gain, feed consumption, feed to gain ratio, marketable carcass weight, muscle tissue weight and yield (breast, thigh, leg, wing), and abdominal fat pad weight were compared among groups. Body weight, weight gain, feed intake and feed to gain ratio was measured or calculated at weekly intervals until the end of the trial.

Statistical evaluation was done by analysis of variance (ANOVA) using the replicates as experimental unit for survival, feed consumption and feed to gain ratio, and using the individual bird measurements for the other parameters.

Overall mortality/culls was 6.4 % (27 birds). Post-mortem analysis showed inflammation of the kidney in seven birds, five of which also displayed splayed legs, myasthenia or lethargy. Deaths were not treatment related.

Final body weight for females and males, respectively, were 2.51 and 2.78 kg in the T25 group, 2.44 and 2.84 kg in the control group, and 2.43 and 2.76 kg in the reference group. The corresponding values for cumulative feed to gain ratio in females and males were 1.75 and 1.72 in the T25 group, 1.79 and 1.69 in the control group, and 1.77 and 1.72 in the reference group. No significant differences were found for final body weight, weight gain, feed intake or feed to gain amongst males and females. However, as would be expected, females had a lower body weight than males. At study termination the subset of 42 randomly selected birds/treatment processed (three birds per cage) for carcass and tissue weights was examined for gross pathology. Three birds were found with enlarged spleens (two males in the T25 group, one male from the control group). Of the remaining 123 birds, carcass characteristics were not significantly different between groups. There was no significant treatment by gender interaction.

Since all diets were designed to deliver the same nutrition, the expectation was that birds in the three groups would show essentially the same performance characteristics. The results confirmed the nutritional value of the maize T25 grain and the absence of unintended effects able to impact on growth at the level tested.

5.1.3. Allergenicity[38]

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 plant.

[37] Additional information September 2012/Stafford M-140202-02-1 (2010).
[38] Technical dossier/Section D7.9 and additional information received in September 2012.

5.1.3.1. Allergenicity assessment of the newly expressed protein

A weight-of-evidence approach is followed, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (Codex Alimentarius, 2009; EFSA, 2006a; EFSA GMO Panel, 2011a).

The *pat* gene originates from *Streptomyces viridochromogenes*, a soil microorganism that is not known to be allergenic.

In the current application (EFSA-GMO-RX-T25), an updated bioinformatic analysis[39] of the amino acid sequence of the PAT protein using the criterion of 35 % identity in a window of 80 amino acids revealed no significant similarities to known allergens. In addition, the applicant performed an analysis[39] searching for matches of eight contiguous identical amino acid sequences between the PAT protein and known allergens which confirmed the outcome of the previous bioinformatic analysis.

The studies on resistance to degradation of the PAT protein by proteolytic enzymes presented in the current application have been previously assessed by the EFSA GMO Panel (2011c, 2012).

The EFSA GMO Panel has evaluated the safety of the PAT protein in the context of several previous applications and no concerns in relation to allergenicity were identified (EFSA, 2005, 2006c, 2007a, b, c, 2008; EFSA GMO Panel, 2011c, 2012).

Based on all the available information, the EFSA GMO Panel considered that there are no indications that the newly expressed PAT protein in maize T25 may be allergenic.

5.1.3.2. Allergenicity assessment of the whole GM plant or crop

According to the EFSA GMO Panel risk assessment guidelines (EFSA, 2006a; EFSA GMO Panel, 2011a), the applicant should test any potential change in the allergenicity of the whole GM plant by comparing the allergen repertoire with that of its appropriate comparator(s), when the plant receiving the introduced gene is known to be allergenic.

Maize has not been considered to be a common allergenic food[40]. The prevalence of food allergy to maize is low and appears to vary with the geographic location (Moneret-Vautrin et al., 1998; Pastorello et al., 2009; Fonseca et al., 2012). At least 23 IgE-binding proteins have been identified in maize, a number of which are recognised as allergens. Sixteen of these proteins have been reported to be stress related, with an LTP (lipid transfer protein) being the most important allergen (Pastorello et al., 2000, 2009; Pasini et al., 2002; Fonseca et al., 2012). In some studies, most individuals with a positive skin prick test or having IgE antibodies against maize were suffering from a respiratory allergy, and only a few displayed a true food allergy following oral challenge with maize products (Jones et al., 1995; Pasini et al., 2002). However, in another study of 27 patients with a claimed history of maize allergy, one-half were found to be challenge positive and thus had a food allergy to maize (Scibilia et al., 2008).

Batista et al. (2005) carried out skin prick tests in 50 individuals (mean age 31 years) with asthma–rhinitis, who were considered highly likely to have consumed products containing GM maize. Protein extracts from GM maize T25 and conventional maize were used, along with a number of other extracts. Four individuals showed a positive skin prick reaction to conventional maize, with similar reactions to GM maize T25. No reactions to GM maize without an accompanying reaction of similar magnitude to conventional maize were observed. However, the study presents some limitations associated, for instance, with the low number of study subjects with a true food allergy to maize (in three of the four sensitive individuals the reactions to maize were likely to be due to cross-reactivity

[39] Additional information received in September 2012.

[40] Directive 2007/68/EC of the European Parliament and of the Council of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ, L310, 11-14.

because of primary sensitisation to grass pollen). Despite the limitations, the study provides valuable supplementary information for the allergenicity assessment of the food/feed derived from maize T25.

In the context of this application, and based on the available information, there is no evidence that the genetic modification might significantly change the overall allergenicity of maize T25.

5.1.4. Nutritional assessment of GM food/feed

The intended trait of maize T25 is herbicide tolerance, with no intention to alter the nutritional parameters. The outcome of the composition analysis (see Section 4.1.2) confirmed the nutritional equivalence of the food and feed products derived from maize T25. The introduction of these products into the food and feed chain is, therefore, expected to have no nutritional impact compared with its conventional counterpart and non-GM maize varieties.

Phipps et al. (2005)[41] divided 60 Holstein cows into four groups in a 12-week feeding study. Each group was fed silage from either maize T25, a near-isogenic non-GM maize or one of two commercial non-GM varieties. These four maize varieties were grown under similar general management conditions. Silage was then prepared from the four maize varieties. They were similar in the nutritive value, fermentation characteristics, mineral content and amino acid composition. The total mixed ration (TMR) contained 390 g silage dry matter per kg total dry matter (DM).

DM intake of TMR was significantly lower for the near-isogenic diet than for the GM maize and the two commercial varieties. No significant differences between groups were observed in milk yield, milk composition, and yield of milk constituents. No tDNA of the event T25 or an endogenous *Zea mays* gene encoding the alcohol dehydrogenase was detected by PCR in 90 milk samples from the maize T25 group collected on weeks 1, 6, and 12 of the study. The PAT protein expressed by the T25 maize was also not detected in milk by ELISA.

Nutritional equivalence indicated by the compositional data was supported by the results of feeding studies with dairy cows (Phipps et al., 2005) and chickens (see Section 5.1.3.3b). The EFSA GMO Panel concluded that maize T25 is as nutritious as other maize varieties commercially available.

5.1.5. Post-market monitoring of GM food/feed

None of the available data indicates that maize T25 is any less safe or nutritious than its conventional counterpart. Therefore, in line with the guidance document (EFSA, 2006a; EFSA GMO Panel, 2011a), the EFSA GMO Panel concluded that post-market monitoring of the GM food/feed is not necessary.

5.2. Conclusion

The EFSA GMO Panel has evaluated the safety of the PAT protein in the context of the present application and several previous applications, and no safety concerns were identified (EFSA, 2005, 2006c, 2007a, b, c, 2008; EFSA GMO Panel, 2011c, 2012).

The compositional data indicating the nutritional equivalence of maize T25 were supported by the results of the feeding studies with dairy cow and chickens. In addition, there is no evidence that the genetic modification might significantly change the overall allergenicity of maize T25.

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

The scope of application EFSA-GMO-RX-T25 is for food and feed uses, import and processing of maize T25 and does not include cultivation. Considering the intended uses of maize T25, the environmental risk assessment is mainly concerned with ingestion by animals, and their manure and

[41] Technical dossier/Phipps (2005).

faeces causing exposure of gastrointestinal tract and soil microorganisms, and with the accidental release into the environment of viable maize T25 grains during transport and/or processing. Maize T25 was developed to express the enzyme phosphinothricin acetyl-transferase (PAT), encoded by the *pat* gene from *Streptomyces viridochromogenes* codon-optimised for expression in plants (see Section 3.1.2). Expression of PAT confers tolerance to glufosinate-based herbicides.

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 of 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).

In accordance with its guidance document on the environmental risk assessment of GM plants (EFSA GMO Panel, 2010), the EFSA GMO Panel follows a weight-of-evidence approach in collating and assessing appropriate information from various data sources (e.g. molecular and compositional data, available agronomic and phenotypic data from field trials performed by the applicant, literature) in order to assess the likelihood of unintended effects on the environment. The applicant provided molecular and compositional data that are assessed by the EFSA GMO Panel in Sections 3 and 4.1.2, respectively. In addition, the applicant presented agronomic and phenotypic data gathered from field trials with maize T25 across two locations in Canada in 1995, four locations in France in 2000 and two locations in Brazil over the 2011–2012 growing season (for further details, see Section 4.1.3).

Maize transformation event T25 occurs in forage maize variety Chardon LL, which has been grown in DUS[42] and VCU[43] variety trials in the UK, which assessed the variety's agronomic and phenotypic characteristics in comparison with commercial varieties. In addition, some agronomic parameters, such as crop height and plant density, were studied in the UK's Farm-Scale Evaluations (Champion et al., 2003; Hawes et al., 2003; Heard et al., 2003a, b; Firbank et al., 2006). Data from variety trials in the UK and by Hawes et al. (2003) showed that early in the season Chardon LL was slightly taller than comparator varieties, and data from variety trials in the UK show slightly later maturity characteristics. However, these differences in height were transient, and Chardon LL was similar in most other characteristics, including biomass yield, to the comparator varieties. Thus, Chardon LL has characteristics typical of other forage maize varieties and there are no indications of any significant changes to its agronomic characteristics. In addition, Crawley et al. (2001) studied the fate of sown individuals, to measure recruitment over multiple seasons in various locations, in order to determine whether the GM maize would be more invasive or more persistent in natural habitats than a non-GM comparator. The authors assessed the GM maize, recording its growth, mortality, flowering and seed set over time, and concluded that there were no indications of changes in the establishment, persistence and invasiveness of the GM maize that would indicate a change in its ferality.

Moreover, the EFSA GMO Panel is not aware of any further scientific report of increased spread and establishment of maize T25 or maize with comparable properties or of any change in survival capacity, including overwintering.

[42] DUS tests = tests to establish if the new variety is clearly distinguishable from all other existing varieties within the crop concerned (**D**istinct), whether the variety remains uniform during propagation (**U**niform) and whether the characteristics of the variety remain stable during repeated propagation (**S**table).
[43] VCU tests = tests to check the **V**alue for **C**ultivation and **U**se of the new variety.

Considering all available information related to the agronomic and phenotypic characterisation of maize T25, the EFSA GMO Panel did not observe any enhanced fitness characteristics of maize T25 that would change its capacity to spread, establish or persist compared with non-GM maize, except in the presence of glufosinate-based herbicides. Therefore, the EFSA GMO Panel concludes that, considering the scope of this application, the weight of available evidence and the poor ability of maize to survive outside cultivated land, there were no indications that maize T25 would differ in environmental impacts if there was accidental release into the environment of viable grains.

6.1.1.2. Potential for gene transfer

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 (for further details, see EFSA, 2009).

A successful horizontal gene 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 (HR). The similarity between the plant and bacterial sequences can be situated in the transgene itself or in the flanking regions. In the case of sequence similarity between the transgenic DNA and the natural variants of the gene in bacteria, recombination could result in a gene replacement in bacteria.

Maize T25 contains the following recombinant DNA sequences: the 35S promoter from *Cauliflower mosaic virus* (CaMV), a *pat* gene from *Streptomyces viridochromogenes* codon-optimised for expression in plants, and the 35S transcription terminator from CaMV (for further details, see Section 3). The modified *pat* gene shows high similarity to a bacterial gene which could theoretically provide sites for homologous recombination and thus promote horizontal gene transfer. Furthermore, the plant DNA contains at the 5′ end of the integrated T25 expression cassette 604 bp of DNA derived from the *Escherichia coli* cloning vector pUC18), which consists of a fragment of the *lacZ* gene. In addition, at the 3′ end of the integrated T25 expression cassette, the plant DNA contains 1 840 bp of the plasmid including a 665-bp fragment of a β-lactam antibiotic resistance gene (*bla* gene) and the origin of replication (*ori*) of the pUC18 (which originates from plasmid pMB1 of an *E. coli* strain used to construct plasmid pBR322).

The modified *pat* gene, optimised for expression in plants, originates from *S. viridochromogenes*, which is a member of the phylum Actinobacteria. Natural variants of the *pat* gene (i.e. *bar*, *hpat*, *mat*) have been found in other bacteria with the capacity to produce the amino acid phosphinothricin (glufosinate), among them the Actinobacteria *Streptomyces hygroscopicus* and *Kitasatospora phosalacinea*. All of these bacteria are considered to be spore-forming soil bacteria and not to be regular inhabitants of the gastrointestinal tract of humans or animals. Owing to the potential for wide environmental distribution of bacterial spores, however, it cannot be excluded that spores of *S.*

viridochromogenes or other Actinobacteria may also occasionally occur in these main receiving environments. Furthermore, outside this main route of exposure through food and feed uses, recombinant DNA of maize T25 may also accidentally come in contact with such Actinobacteria in soil. Therefore, various routes of exposure were 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 T25 or any other GM plant), it can be assumed that, as an extremely rare event, HR may occur between the recombinant *pat* gene and their natural variants (i.e. *pat*, *bar*, *hpat*, *mat*) in the environments described above.

The DNA sequence similarity between the plasmid region including the origin of replication (length 1 177 bp) and the β-lactam antibiotic resistance gene fragment (length 665 bp) and the corresponding genes in natural plasmids of *E. coli*, and possibly other Enterobacteriaceae, potentially facilitates HR between the transgenic DNA sequences and those plasmids as they may occur in *E. coli* and other Enterobacteriaceae. In fact, *E. coli* and other Enterobacteriaceae are present in the main receiving environment, i.e. the gastrointestinal tract. However, because of the molecular structure (direct vicinity of both sequences), such a recombination process would not result in the acquisition of an additional gene and therefore no novel selective advantage would be provided.

Theoretically, double homologous recombination between the oriV and/or *bla* sequences and the *lacZ* sequences could result in the insertion of the *pat* gene onto pUC/pBR322-like plasmids of *E. coli* (cloning vectors) or into the chromosome. The chromosomal recombination is unlikely to result in viable bacteria with a *pat* gene since the double homologous recombination would cause a deletion of other chromosomal genes located between the two genetic elements (*bla* and *lacZ* sequences). Only cloning vectors, i.e. pUC or pBR322 with a *bla* gene, could function as recipient molecules, if they occurred as contaminants in the environment. Therefore, scenarios in which the *pat* gene would be transferred from DNA of maize T25 to synthetic resistance plasmids were also considered here in assessing the risk for horizontal gene transfer (see below).

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 *pat* gene and the prevalence of bacterial genes encoding for the enzyme phosphinothricin acetyl-transferase in the environment, a low-level gene transfer to *S. viridochromogenes* or other bacterial species, including a transfer onto plasmids, is thought not to confer a new trait and selective advantage. Considering its intended uses 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 T25 to bacteria.

b) Plant-to-plant gene transfer

Considering the intended uses of maize T25 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 transport and/or 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 transport and/or 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 cultivation in Europe is mainly limited by a combination of low competitiveness, 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 T25 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 extremely low. In conclusion, considering the scope of this application, a weight-of-evidence approach 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 adverse 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 T25, excluding cultivation, and the absence of target organisms, potential interactions of the GM plant with target organisms were not considered an issue by the EFSA GMO Panel.

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

Owing to the intended uses of maize T25, 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 T25, 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 post-market environmental monitoring (PMEM) 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 PMEM plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b). The potential exposure to the environment of maize T25 would be mainly through ingestion by animals, and their manure and faeces leading to exposure of gastrointestinal tract and soil microorganisms, and with the accidental release

into the environment of viable maize T25 grains during transport and/or processing. The scope of the PMEM 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 T25, no case-specific monitoring is required.

The PMEM 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 PMEM report on an annual basis.

The EFSA GMO Panel is of the opinion that the scope of the PMEM plan proposed by the applicant is in line with the intended uses of maize T25 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 PMEM plan.

6.3. Conclusion

Considering the intended uses of maize T25, the environmental risk assessment is concerned with indirect exposure, mainly through ingestion by animals, and their manure and faeces resulting in exposure of gastrointestinal tract and soil microorganisms, and with the accidental release into the environment of viable maize T25 grains during transport and/or processing.

Considering all available information related to the agronomic and phenotypic characterisation of maize T25, the EFSA GMO Panel did not observe any enhanced fitness characteristics of maize T25 that will change its capacity to spread, establish or persist compared with non-GM maize, except in the presence of glufosinate-based herbicides. 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 T25 to bacteria have not been identified. The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of maize T25 and the guidance document of the EFSA GMO Panel on PMEM of GM plants (EFSA GMO Panel, 2011b). The EFSA GMO Panel agreed with the reporting intervals proposed by the applicant in the PMEM plan.

7. Overall conclusion for application EFSA-GMO-RX-T25

The molecular characterisation data established that the genetically modified maize T25 contains a single insertion locus containing a *pat* cassette. Bioinformatic analyses, protein expression data and genetic stability studies did not raise safety issues.

The compositional, agronomic and phenotypic characteristics of grain maize T25 and its conventional counterpart showed no differences of relevance for food/feed safety. The EFSA GMO Panel has evaluated the safety of the PAT protein in the context of the present application and several previous applications, and no safety concerns were identified. Data from nutritional studies with whole food/feed did not raise any safety concern. There was no evidence that the genetic modification might significantly change the overall allergenicity of maize T25.

The environmental risk assessment did not identify evidence of any adverse environmental impacts due to the accidental release into the environment of viable grains from maize T25. No data indicating adverse impacts to human/animal health and the environment have emerged since the authorisation of maize T25.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of maize T25 and the guidance document of the EFSA GMO Panel on PMEM of GM plants (EFSA GMO Panel, 2011b). In addition, the EFSA GMO Panel acknowledged 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 T25. The EFSA GMO Panel agreed with the reporting intervals proposed by the applicant in the PMEM plan.

In conclusion, the EFSA GMO Panel considered that the information available for maize T25 confirms the previous opinions on its safety (SCP, 1998; ACNFP, 2003) and allows the Panel to conclude that maize T25 is as safe as its conventional counterpart with respect to potential effects on human and animal health or the environment in the context of its intended uses.

8. Risk assessment and overall conclusion for application EFSA-GMO-NL-2007-46

The scope of application EFSA-GMO-NL-2007-46 includes food containing or consisting of maize T25 in addition to the scope of EFSA-GMO-RX-T25 (food and food ingredients produced from maize T25 and feed containing, consisting of or produced from maize T25). Considering the scope of the two applications, the risk assessment and conclusions drawn for maize T25 in EFSA-GMO-RX-T25 are valid for maize T25 in the context of application EFSA-GMO-NL-2007-46. Therefore, the EFSA GMO Panel considered that maize T25, as described in application EFSA-GMO-NL-2007-46, is as safe as its conventional counterpart with respect to potential effects on human and animal health or the environment in the context of its intended uses.

OVERALL CONCLUSIONS

In conclusion, the EFSA GMO Panel considered that maize T25, as described in applications EFSA-GMO-RX-T25 and EFSA-GMO-NL-2007-46, is as safe as its conventional counterpart with respect to potential effects on human and animal health or the environment in the context of its intended uses for food and feed, import and processing.

DOCUMENTATION PROVIDED TO EFSA IN RELATION TO EFSA-GMO-RX-T25

1. Letter from the European Commission, received on 29 June 2007, concerning a request for renewal of authorisation for the placing on the market of genetically modified maize T25 submitted in accordance with articles 8(1)(a) and 20(1)(a),(b) of Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 20 July 2007, from EFSA to the European Commission.

3. Letter from EFSA to applicant, dated 7 March 2008, requesting additional information under completeness check.

4. Letter from applicant to EFSA, received on 3 October 2008, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 9 October 2008, delivering the "Statement of Validity" for application EFSA-GMO-RX-T25, regarding genetically modified maize T25 submitted under Regulation (EC) No 1829/2003 by Bayer.

6. Letter from EFSA to applicant, dated 9 October 2008, concerning EFSA-GMO-NL-2007-46 and EFSA-GMO-RX-T25 and stopping the clock.

7. Letter from applicant to EFSA, received on 1 September 2010, providing additional information concerning T25.

8. Letter from EFSA (UK CA) to applicant, dated 8 September 2010, restarting the clock.

9. Letter from EFSA to applicant, dated 29 March 2012, requesting additional information and stopping the clock.

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

11. Letter from EFSA to applicant, dated 11 July 2012, concerning finalisation of the EFSA assessment.

12. Letter from applicant to EFSA, dated 20 August 2012, concerning finalisation of the EFSA assessment.

13. Letters from applicant to EFSA, received on 3 September 2012, providing additional information.

14. Letter from EFSA to applicant, dated 15 October 2012, concerning finalisation of the EFSA assessment.

15. Letter from EFSA to applicant, dated 18 October 2012, restarting the clock.

16. Letter from applicant to EFSA, received on 18 December 2012, providing additional information.

17. Letter from applicant to EFSA, received on 14 January 2013, concerning change of scope.

18. Letter from EFSA to applicant, dated 4 February 2013, requesting additional information and stopping the clock.

19. Letter from applicant to EFSA, received on 19 February 2013, providing additional information.

20. Letter from EFSA to applicant, dated 27 May 2013, restarting the clock.

21. Letter from applicant to EFSA, received on 29 May 2013, indicating availability to provide additional information.

22. Letter from EFSA to applicant, dated 30 May 2013, referring to letter sent on 11 July 2012.

23. Letter from applicant to EFSA, received on 26 June 2013, providing spontaneous additional information.

DOCUMENTATION PROVIDED TO EFSA IN RELATION TO EFSA-GMO-NL-2007-46

1. Letter from the Competent Authority of the Netherlands, received on 24 April 2007, concerning a request for the placing on the market of genetically modified maize T25 submitted under Regulation (EC) No 1829/2003 by Bayer.

2. Acknowledgement letter, dated 12 July 2007, from EFSA to the Competent Authority of the Netherlands.

3. Letter from EFSA to applicant, dated 4 October 2007, requesting additional information under completeness check.

4. Letter from applicant to EFSA, received on 23 May 2008, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 10 June 2008, delivering the "Statement of Validity" for application EFSA-GMO-NL-2007-46, regarding genetically modified maize T25 submitted under Regulation (EC) No 1829/2003 by Bayer.

6. Letter from EFSA (UK CA) to applicant, dated 1 October 2008, requesting additional information and stopping the clock.

7. Letter from EFSA to applicant, dated 9 October 2008, concerning EFSA-GMO-NL-2007-46 and EFSA-GMO-RX-T25.

8. Letter from applicant to EFSA (UK CA), received on 1 July 2009, providing additional information.

9. Letter from EFSA (UK CA) to applicant, dated 24 June 2010, restarting the clock.

10. Letter from applicant to EFSA, received on 1 September 2010, providing additional information concerning T25.

11. Letter from applicant to EFSA, received on 7 October 2010, providing additional information concerning T25.

12. Letter from EFSA to applicant, dated 29 March 2012, requesting additional information and stopping the clock.

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

14. Letter from EFSA to applicant, dated 11 July 2012, concerning finalisation of the EFSA assessment.

15. Letter from applicant to EFSA, dated 20 August 2012, concerning finalisation of the EFSA assessment.

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26. Letter from EFSA to applicant, dated 30 May 2013, referring to letter sent on 11 July 2012.

23. Letter from applicant to EFSA, received on 26 June 2013, providing spontaneous additional information.

REFERENCES

ACNFP, 1997. Report on processed products from genetically modified glufosinate ammonium tolerant maize (line T25). UK ACNFP, 14 February 1997.

ACNFP, 2003. ACNFP additional data on the nutritional impact assessment of T25 maize. http://www.food.gov.uk/multimedia/webpage/110684

Batista R, Nunes B, Carmo M, Cardoso C, Sao Jose H, de Almeida AB, Manique A, Bento L, Ricardo CP and Oliveira MM, 2005. Lack of detectable allergenicity of transgenic maize and soya samples. Journal of Allergy and Clinical Immunology, 116, 403-410.

Champion GT, May MJ, Bennett S, Brooks DR, Clark SJ, Daniels RE, Firbank LG, Haughton AJ, Hawes C, Heard MS, Perry JN, Randle Z, Rossall MJ, Rothery P, Skellern MP, Scott RJ, Squire GR and Thomas MR, 2003. Crop management and agronomic context of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Philosophical Transactions of the Royal Society B: Biological Sciences, 358, 1801-1818.

Codex Alimentarius, 2009. Foods derived from modern biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme. Rome, Italy, 85 pp.

Crawley MJ, Brown SL, Hails RS, Kohn DD and Rees M, 2001. Biotechnology: transgenic crops in natural habitats. Nature, 409, 682-683.

Eastham K and Sweet J, 2002. Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. European Environment Agency, Environmental Issue Report, 28, 1-75. Available online: http://www.eea.europa.eu/publications/environmental_issue_report_2002_28

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Genetically Modified Organisms on an application (reference EFSA-GMO-NL-2004-02) for the placing on the market of insect-tolerant genetically modified maize 1507, for food use, under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International/Mycogen Seeds. The EFSA Journal 2005, 182, 1-22.

EFSA (European Food Safety Authority), 2006a. Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. The EFSA Journal 2006, 99, 1-100.

EFSA (European Food Safety Authority), 2006b. Guidance document for the renewal of authorisations of existing GMO products lawfully placed on the market, notified according to Articles 8 and 20 of Regulation (EC) No 1829/2003. The EFSA Journal 2006, 435, 1-4.

EFSA (European Food Safety Authority), 2006c. Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-NL-2005-13) for the placing on the market of glufosinate-tolerant genetically modified LLCotton25, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience. The EFSA Journal 2006, 429, 1-19.

EFSA (European Food Safety Authority), 2007a. Opinion of the Scientific Panel on Genetically Modified Organisms on an application (reference EFSA-GMO-UK-2004-04) for the placing on the market of glufosinate tolerant genetically modified rice LLRICE62 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 from Bayer CropScience GmbH. The EFSA Journal 2007, 588, 1-25.

EFSA (European Food Safety Authority), 2007b. Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-NL-2005-12) for the placing on the market of insect-resistant genetically modified maize 59122, for food and feed uses, import and processing under Regulation (EC) No 1829/2003, from Pioneer Hi-Bred International, Inc. and Mycogen Seeds, c/o Dow Agrosciences LLC. The EFSA Journal 2007, 470, 1-25.

EFSA (European Food Safety Authority), 2007c. Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-NL-2005-18) for the placing on the

market of the glufosinate tolerant soybean A2704-12, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience. The EFSA Journal 2007, 524, 1-22.

EFSA (European Food Safety Authority), 2008. Opinion of the Scientific Panel on Genetically Modified Organisms on applications (references EFSA-GMO-UK-2005-25 and EFSA-GMO-RXT45) for the placing on the market of the glufosinate-tolerant genetically modified oilseed rape T45, for food and feed uses, import and processing and for renewal of the authorisation of oilseed rape T45 as existing product, both under Regulation (EC) No 1829/2003 from Bayer CropScience. The EFSA Journal 2008, 635, 1-22.

EFSA (European Food Safety Authority), 2009. Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants. The EFSA Journal 2009, 1108, 1-8.

EFSA Panel on Genetically Modified Organisms (GMO), 2010. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879, 111 pp. doi:10.2903/j.efsa.2010.1879

EFSA Panel on Genetically Modified Organisms (GMO), 2011a. Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011;9(5):2150, 37 pp. doi:10.2903/j.efsa.2011.2150

EFSA Panel on Genetically Modified Organisms (GMO), 2011b. Scientific Opinion on guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. EFSA Journal 2011;9(8):2316, 40 pp. doi:10.2903/j.efsa.2011.2316

EFSA Panel on Genetically Modified Organisms (GMO), 2011c. Scientific Opinion on application (EFSA-GMO-NL-2008-52) for the placing on the market of herbicide tolerant genetically modified soybean A5547-127 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience. EFSA Journal 2011;9(5):2147, 27 pp. doi:10.2903/j.efsa.2011.2147

EFSA Panel on Genetically Modified Organisms (GMO), 2012. Scientific Opinion on application (EFSA-GMO-BE-2010-81) for the placing on the market of genetically modified herbicide-tolerant oilseed rape Ms8, Rf3 and Ms8 × Rf3 for food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape Ms8, Rf3 and Ms8 × Rf3 (with the exception of processed oil) under Regulation (EC) No 1829/2003 from Bayer. EFSA Journal 2012;10(9):2875, 32 pp. doi:10.2903/j.efsa.2012:2875

Firbank LG, Rothery P, May MJ, Clark SJ, Scott RJ, Stuart RC, Boffey CWH, Brooks DR, Champion DT, Haughton AJ, Hawes C, Heard MS, Dewar AM, Perry JN and Squire GR, 2006. Effects of genetically modified herbicide-tolerant cropping systems on weed seedbanks in two years of following crops. Biology Letters 2, 140-143.

Fonseca C, Planchon S, Renaut J, Oliveira MM and Batista R, 2012. Characterization of maize allergens-MON810 vs. its non-transgenic counterpart. Journal of Proteomics, 75, 2027-2037.

Gruber S, Colbach N, Barbottin A and Pekrun C, 2008. Post-harvest gene escape and approaches for minimizing it. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 3, 1-17.

Hawes C, Haughton AJ, Osborne JL, Roy DB, Clark SJ, Perry JN, Rothery P, Daniels D, Young MW, Parish AM, Scott RJ, Firbank LG and Squire GR, Bohan A, Brooks DR, Champion GT, Dewar AM, Heard MS, Woiwod IP, Daniels RE, Young MW, Parish AM, Scott RJ, Firbank LG and Squire GR, 2003. Responses of plants and invertebrate trophic groups to contrasting herbicide

regimes in the Farm Scale Evaluations of genetically modified herbicide tolerant crops. Philosophical Transactions of the Royal Society B: Biological Sciences, 358, 1899-1913.

Heard MS, Hawes C, Champion GT, Clark SJ, Firbank LG, Haughton AJ, Parish A, Perry JN, Rothery P, Scott RJ, Skellern M, Squire GR and Hill MO, 2003a. Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. 1. Effects on abundance and diversity. Philosophical Transactions of the Royal Society B: Biological Sciences 358, 1819-1832.

Heard MS, Hawes C, Champion GT, Clark SJ, Firbank LG, Haughton AJ, Parish AM, Perry JN, Rothery P, Roy DB, Scott RJ, Skellern MP, Squire GR and Hill MO, 2003b. Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. 2. The effects on individual species. Philosophical Transactions of the Royal Society B: Biological Sciences, 358, 1833-1846.

Hülter N and Wackernagel W, 2008. Double illegitimate recombination events integrate DNA segments through two different mechanisms during natural transformation of *Acinetobacter baylyi*. Molecular Microbiology, 67, 984-995.

Jones SM, Magnolfi CF, Cooke SK and Sampson HA, 1995. Immunological cross-reactivity among cereal-grains and grasses in children with food hypersensitivity. Journal of Allergy and Clinical Immunology, 96, 341-351.

Kim CG, Yi H, Park S, Yeon JE, Kim DY, Kim DI, Lee KH, Lee TC, Paek IS, Yoon W, Jeong SC and Kim HM, 2006. Monitoring the occurrence of genetically modified soybean and maize around cultivated fields and at a grain receiving port in Korea. Journal of Plant Biology, 49, 218-298.

Lecoq E, Holt K, Janssens J, Legris G, Pleysier A, Tinland B and Wandelt C, 2007. General surveillance: roles and responsibilities: the industry view. Journal für Verbraucherschutz und Lebensmittelsicherheit–Journal of Consumer Protection and Food Safety, 2, 25-28.

Lee B, Kim C, Park J-Y, Park K, Kim H-J, Yi H, Jeong C-C, Yoon W and Kim H, 2009. Monitoring the occurrence of genetically modified soybean and maize in cultivated fields and along the transportation routes of the Incheon Port in South Korea. Food Control, 20, 250-254.

Moneret-Vautrin DA, Kanny G, Beaudouin E, 1998. Food allergy to corn—does it exist? Allergie et immunologie, 30, 230-230.

NRC (National Research Council), 1994. Nutrient Requirements of Poultry, 9th revised edition, National Research Council, Washington, D.C, 176 pp.

OECD (Organisation for Economic Cooperation and Development), 2002. Consensus document on compositional considerations for new varieties of maize (Zea mays): Key food and feed nutrients, anti-nutrients and secondary plant metabolites. Available online: http://www.oecd.org/science/biotrack/46815196.pdf.

OECD (Organisation for Economic Cooperation and Development), 2003. Consensus document on the biology of Zea mays subsp. mays (Maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2003)11), No 27, 1-49. Available online: http://www.olis.oecd.org/olis/2003doc.nsf/LinkTo/NT0000426E/$FILE/JT00147699.PDF

Palaudelmàs M, Penas G, Mele E, Serra J, Salvia J, Pla M, Nadal A and Messeguer J, 2009. Effect of volunteers on maize gene flow. Transgenic Research, 18, 583-594.

Park KW, Lee B, Kim CG, Kim D-Y, Park J-Y, Ko EM, Jeong S-C, Choi KH, Yoon WK and Kim HM, 2010. Monitoring the occurrence of genetically modified maize at a grain receiving port and along transportation routes in the Republic of Korea. Food Control, 21, 456-461.

Pasini G, Simonato B, Curioni A, Vincenzi S, Cristaudo A, Santucci B, Peruffo ADB and Giannattasio M, 2002. IgE-mediated allergy to corn: a 50 kDa protein, belonging to the Reduced Soluble Proteins, is a major allergen. Allergy, 57, 98-106.

Pastorello E, Farioli L, Pravettoni V, Ispano M, Scibola E, Trambaioli C, Giuffrida MG, Ansaloni R, Godovac-Zimmermann J, Conti A, Fortunato D and Ortolani C, 2000. The maize major allergen,

which is responsible for food-induced allergic reactions, is a lipid transfer protein. Journal of Allergy and Clinical Immunology, 106, 744-751.

Pastorello E, Farioli L, Pravettoni V, Scibilia J, Conti A, Fortunato D, Borgonovo L, Bonomi S, Primavesi L and Ballmer-Weber B, 2009. Maize food allergy: lipid-transfer proteins, endochitinases, and alpha-zein precursor are relevant maize allergens in double-blind placebo-controlled maize-challenge-positive patients. Analytical and Bioanalytical Chemistry, 395, 93-102.

Scibilia J, Pastorello EA, Zisa G, Ottolenghi A, Ballmer-Weber B, Pravettoni V, Scovena E, Robino A and Ortolani C, 2008. Maize food allergy: a double-blind placebo-controlled study. Clinical and Experimental Allergy, 38, 1943-1949.

SCP, 1998. Opinion of the Scientific Committee on Plants Regarding "Submission for Placing on the Market of Glufosinate Tolerant Corns (*Zea Mays*) Transformation Event T25" by the Agrevo Company. Available online: http://ec.europa.eu/food/fs/sc/scp/out04_en.html

Windels P, Alcalde E, Lecoq E, Legris G, Pleysier A, Tinland B and Wandelt C, 2008. General surveillance for import and processing: the EuropaBio approach. Journal of Consumer Protection and Food Safety, 3(S2), 14-16.