

Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference CE/ES/00/01) for the placing on the market of herbicide-tolerant genetically modified maize NK603, for import and processing, under Part C of Directive 2001/18/EC from Monsanto¹ (Question No EFSA-Q-2003-003)

Opinion adopted on 25 November 2003

SUMMARY

This document provides an opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on genetically modified maize NK603 and derived food and feed products. The opinion is based on two questions raised by the Commission related to applications for the placing of the maize on the market by Monsanto under the Novel Food Regulation (EC) No 258/97 and the Directive 2001/18/EC on the deliberate release of genetically modified organisms (GMOs) into the environment (EC, 1997; EC, 2001).

In the first question, the GMO Panel was asked to consider the safety of foods and food ingredients derived from NK 603 maize and in the second question it was requested to consider whether there is any scientific reason to believe that the placing on the market of NK603 maize, for import and processing, is likely to cause any adverse effects on human health and the environment. The questions followed two separate scientific assessments which were initially made by the appropriate authorities in The Netherlands and Spain and subsequently evaluated by all other Member States. An assessment of the NK603 maize was requested by the Commission because of questions raised by several Member States following the evaluations at national level. When this is the case, EU legislation requires that EFSA carries out a further assessment and provides an opinion.

In delivering its opinion the Panel considered the applications and additional information provided by the applicant and the specific questions and concerns raised by the Member States. At the request of the Commission, the Panel has provided two separate opinions. However, as both dossiers cover to a large extent the same issues a single risk assessment is provided for both opinions.

The risk assessment process was conducted using scientific guidance published by the EC Scientific Committees (EC, 2003). It included examination of the DNA integrated into NK603 using particle bombardment, the nature and safety of the target proteins produced by the transgenic event and the possibility that the genetic modification may have influenced the safety (including allergenicity) and the nutritional value of NK603 in comparison with conventional maize.

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The NK603 maize has been genetically modified to provide tolerance to the herbicide glyphosate. The stated purpose of the modification is to allow farmers to manage weeds more effectively in maize fields during cultivation. NK603 maize has been planted for field studies within the EU and has been marketed in several countries outside the EU. The present applications concern only import and processing, but not cultivation of the maize. If approved it would therefore make it possible to place on the market NK603 maize and derived products, such as starch, oil, maize gluten feed and maize meal for food and feed use, whereas the crop would be grown and harvested outside the EU.

Glyphosate tolerance was achieved by the introduction of a gene encoding glyphosate tolerant 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). The EPSPS activity is needed for the biosynthesis of aromatic amino acids in plants and in micro-organisms but the structure of the plant enzyme makes it commonly vulnerable to glyphosate, thereby causing the plants to be killed by the herbicide.

Molecular analysis showed that NK603 contains a single inserted copy of the DNA present in the construct used for the transformation. The plasmid vector contains two adjacent plant gene expression cassettes each containing a single copy of the *cp4 epsps* gene. The insert in NK603 does include some molecular re-arrangements at one end of the insert and also includes a fragment of chloroplast DNA. These re-arrangements and the insertion of chloroplast DNA do not lead to new traits and are not considered to pose a safety risk. In the unlikely event that a new peptide or protein is produced as a consequence of the insertion event, bioinformatics analysis showed that these would have no homology to known toxins or allergens.

As a result of the genetic modification NK603 contains two slightly different CP4 EPSPS proteins expressed from two copies of the *cp4 epsps* gene using different promoters. The proteins differ from each other in one amino acid. Analysis of the impact of this change indicated no apparent changes in EPSPS protein structure, activity, toxicity or allergenicity using appropriate bioinformatics approaches, *in vitro* digestion procedures and studies on experimental animals. Furthermore, appropriate animal feeding trials indicated that NK603 was as safe as its non-GM comparator. Analysis of the grain from field trials in the USA and Europe showed that NK603 had the same composition as its non-GM comparator.

The notification C/ES/00/01 for maize NK603 only concerns import. There is therefore no requirement for scientific information on possible environmental effects associated with the cultivation of maize NK603. The GMO Panel agrees with the conclusions of the environmental risk assessment by the applicant that the likelihood of unintended environmental effects due to the adventitious release and spread of NK603 maize will not be different from that of traditionally bred maize. The monitoring plan provided by the applicant is in line with the intended uses for the GMO.

In conclusion, the Panel has considered all the evidence provided and is of the opinion that NK603 maize is as safe as conventional maize and therefore the placing on the market of NK603 maize for food or feed or processing is unlikely to have an adverse effect on human or animal health or, in that context, on the environment.

Key words: GMOs, maize NK603, herbicide tolerance, glyphosate, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), food safety, feed safety, human health, environment, import, Regulation (EC) 258/97, Directive 2001/18/EC.

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BACKGROUND

The Commission received the notification for the placing on the market of glyphosate-tolerant genetically modified (GM) maize NK603, for import and processing, under part C of Directive 2001/18/EC² (Reference CE/ES/00/01), from Monsanto, on 17 January 2003, following a positive assessment from the lead Member State (Spain).

In accordance with Directive 2001/18/EC, the notification was then transmitted to the competent authorities of other Member States, a number of which have raised objections during the statutory 60-day period.

Where these objections cannot be resolved within the subsequent 45-day period, the Commission is required to consult the relevant Scientific Committees for opinion. The European Food Safety Authority is considered to be the relevant Scientific Committee for this purpose.

TERMS OF REFERENCE

Article 18(1) of Directive 2001/18/EC states that the period of time during which the Commission is awaiting the opinion of the Scientific Committee shall not exceed 90 days. EFSA is therefore requested, under Article 29(1) and in accordance with Article 22(5)(c) of Regulation (EC) No 178/2002³ (EC, 2002), to provide a scientific opinion, as soon as possible and at the latest by the end of November 2003, as to whether there is any scientific reason to believe that the placing on the market of the NK603 maize, for import and processing, is likely to cause any adverse effects on human health and the environment within the scope of Directive 2001/18/EC.

² 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.

³ Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

In particular, EFSA is requested to take account of the objections raised by the competent authorities of Member States in this context.

EFSA is not requested to give an opinion on the non-scientific objections raised by Member States, in the context of the entry into force of forthcoming legislation or requests for further legislative/implementing measures.

ASSESSMENT

1. Molecular characterisation

1.1 The transformation process and vector constructs

Maize line AW x CW, used in the initial transformation, is a proprietary maize cell culture, which was transformed using particle acceleration technology to develop the NK603 maize event. Embryogenic maize cells of AW x CW were, therefore, the initial recipient of the introduced DNA. Conventional breeding methods were used to backcross plants generated from the initial transformation into a recurrent, desired inbred maize line with a genetic background of interest to the breeder.

NK603 has been developed for tolerance to glyphosate by the introduction of a gene coding for glyphosate tolerant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). Particle acceleration was used to introduce a fragment of DNA isolated from the bacterial plasmid vector PV-ZMGT32. The plasmid vector contains two adjacent plant gene expression cassettes each containing a single copy of the *cp4 epsps* gene fused to chloroplast transit peptide (CTP) sequences based on sequences derived from *Arabidopsis thaliana* EPSPS. CTP targets the CP4 EPSPS protein to its natural sub cellular location in the chloroplast. In the first *ctp2-cp4 epsps* cassette the coding sequence is regulated by the rice actin promoter and a rice intron sequence introduced upstream of the CTP sequence. Expression of the second *ctp2-cp4 epsps* cassette is regulated by an enhanced 35S CaMV promoter and a maize intron derived from a gene encoding a heat shock protein. In each cassette the *cp4 epsps* sequence is linked to the nopaline synthase terminator (NOS 3') sequence from *Agrobacterium tumefaciens*. The vector also contains an *nptII* bacterial selectable marker gene (for kanamycin resistance; derived from the prokaryotic transposon *Tn5*) and an origin of replication (*ori*). A *MluI* restriction fragment of the PV-ZMGT32 plasmid vector, designated PV-ZMGT32L, was used for transformation and this fragment only contains the *cp4 epsps* plant gene expression cassettes. The *nptII* gene as well as the *ori* are not present in the fragment PV-ZMGT32L.

Use of the 35S CaMV promoter

The 35S CaMV promoter is derived from the common cauliflower mosaic virus (CaMV) and is a promoter frequently used in the genetic modification of (crop) plants. It has been suggested (Ho et al., 1999) that the 35S CaMV promoter could result in an inadvertent activation of plant genes or endogenous viruses, promote horizontal gene transfer, or might even recombine with mammalian viruses with unexpected consequences. The UK Advisory Committee on Releases of GM crops into the Environment has considered the 35S CaMV promoter issue (ACRE, 2002) and concluded that no new data or direct experimental evidence has been presented to support the hypothesis that the promoter is inherently unsafe. Moreover, the Committee emphasized that humans and animals have been eating plant material containing the 35S promoter via natural

CaMV infection and no adverse effects have been reported. The Panel concurs with ACRE's comments. Additional arguments for the safety of the promoter in GM crops are provided by Hull et al. (2000).

1.2 Transgenic constructs in the genetically modified plant

The molecular characterisation of NK603 showed that both *ctp2-cp4 epsps* gene cassettes are intact within NK603. The sequence of the *cp4 epsps* gene from the first cassette is identical in NK603 to that in the original plasmid, whilst in the second inserted cassette the sequence of the *cp4 epsps* gene differs by 2 nucleotides from that in the original plasmid (due to this difference the gene is designated *cp4 epsps* L214P). These nucleotide changes result in one silent mutation and one amino acid substitution of proline for leucine at amino acid position 214.

Southern blotting and PCR have been used to provide data on inserts within the derived GM line NK603. The analysis included the use of appropriate restriction endonucleases and showed that the insert is a single complete copy of PV-ZMGT32L. There is no detectable presence of plasmid DNA from outside of the left and right borders of plasmid vector PV-TMGT32L. The insert also includes an inversely linked 217 bp DNA fragment of the enhancer region of the rice actin promoter (at the 3' end). This fragment does not contain sequences needed for promoter activity. Next to this 217 bp fragment is a 305 bp region with homology to chloroplast DNA. Both 3' and 5' ends of the insert were confirmed by PCR and DNA sequencing and the sequences flanking the insert are confirmed as belonging to the maize genome. Sequences of 307 and 497 bp were provided, respectively, for the 5' and 3' flanking regions of the NK603 genome.

Some Member States have asked for additional experimental data on the presence or absence of additional chloroplast or mitochondrial DNA in the nuclear DNA of NK 603 maize. Multiple independent gene transfers from the mitochondria and chloroplasts to the nuclear genome have been reported in the scientific literature. Functional gene establishment of organellar DNA in the nucleus is rare, but DNA transfer without functionality is presumed to be more frequent. For example, in tobacco DNA is transferred from the chloroplast and integrated into the nucleus at a frequency of one in approximately 16,000 pollen grains (Huang et al., 2003; minimum estimate). Indeed, long tracts of organellar DNA are found in the eukaryotic chromosomes of plants. For example, about 620 kb of mitochondrial DNA has been found on chromosome 2 of *Arabidopsis*, and about 33 kb of chloroplast DNA on chromosome 10L of rice. Furthermore, eukaryotes generally contain DNA sequences in their nuclear genome that show close similarity to organellar DNA, suggesting that transfer of chloroplast and mitochondrial DNA are ongoing processes. The Panel is of the opinion that there is no reason to suggest that any genes and gene products of maize organelles in NK603 maize carry any increased risk potential compared with the organellar genes in the non-GM comparator. Specifically for NK603, and concerning the chloroplast fragment inserted, the Panel considers that data provided from bioinformatic analysis and other safety studies address the issue of potential unintended effects caused by insertion of the fragment. The Panel concludes that insertion of the chloroplast fragment in NK603 does not constitute a risk.

Reverse transcriptase PCR analysis (RT PCR) was used to determine whether transcripts are produced which encompass the 3' end of the insert (which includes rice actin promoter sequences and chloroplast DNA fragments) and the adjacent 3' flanking region of maize genomic DNA. RT PCR did detect a transcription product which initiates within the NK603 insert (in the actin or 35S CaMV promoter regions) and which is processed through the NOS 3' terminator into the maize genome flanking 3' region. This could create 2 or more mRNA species, a smaller one at 1.4 kb (predicted as the *cp4 epsps* L214p transcript) and a larger species at >1.4kb (a product likely to be the result of incomplete termination at the NOS 3' genetic element due to "read through"). The RT PCR revealed that the larger fragment was a

very small fraction of transcript produced from the *cp4 epsps* L214p insert. Transcription of the larger fragment was undetectable in Northern blots using probes based on elements downstream of NOS 3'. As predicted, the 1.4 kb element was detectable in Northern blots using a *cp4* gene probe.

“Read through” transcription is routinely observed in many plant genes. Furthermore, the RNA fragment observed in the product of the RT PCR amplification is not expected to have a regulatory function as described for micro RNAs which are short RNAs between 21 and 23 nt long derived from the processing of longer RNAs of around 70 nt (Jones, 2002). This is much shorter than the RNA fragments amplified from NK603.

1.3 Inheritance and stability of inserted DNA

Segregation data for nine generations of line NK603 have demonstrated the stability of the inserted DNA through six generations of crossing and three generations of self-pollination.

2. Comparative Analysis

2.1 Choice of the comparator

LH82 x B73 (sometimes abbreviated as “B73”) maize can be considered to be an appropriate control hybrid for NK603 maize as it has a genetic background which is comparable to that of the NK603 event used in the molecular and compositional analyses, but lacks the genes encoding the CP4 EPSPS proteins. The applicant confirms that LH82 x B73 is a non-GM control hybrid used in the molecular analyses of NK603 maize, as well as in the compositional analyses performed on material grown in the US and Europe. These analyses have been included in the application for NK603 maize. The flanking sequence research on NK603 maize, which made use of a B73 control line, has established that the flanking sequences of NK603 maize are related to the B73 non-GM control and are native to the maize genome. Thus B73 can be considered as an appropriate control line for comparative evaluations.

2.2 Field trials and compositional analysis

The material to be analysed in the comparative analysis was collected from field trials in the USA (year 1998) and Europe (year 1999). The US field trials in 1998 were conducted in Iowa, Illinois, Indiana, and Ohio (two replicated and six non-replicated trials). The field trials in Europe in 1999 were carried out in France and Italy (four replicated trials).

With the exception of the glyphosate-tolerance, NK603 maize is morphologically and agronomically similar to the non-GM comparator. With regard to compositional equivalence a total of 51 different parameters (proximate analysis, specific compounds and groups of compounds) were analysed including ash, carbohydrates, fibre, moisture, protein, total fat, amino- and fatty acids, minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn), vitamin E, and trypsin inhibitor. The levels of different chemical constituents in NK603 maize were either within the range found for the non-transgenic controls or within the ranges reported in published literature. Statistical analysis of the results revealed one significant difference in 1998, for stearic acid in kernels. This difference was minor (NK603: 1.95%; control: 1.86%) and was not observed in 1999. The biological significance of differences was further evaluated by performing additional comparisons of the level of specified compounds in NK603 with the levels in either commercial non-GM maize lines grown in 1998 or two control lines grown in trials conducted in 1994-1995. No conclusive differences requiring further studies were found. Thus NK603 maize used for

food, feed and processing can be considered to have the same composition as the genetically related non-GM maize lines for food and feed use.

3. Environmental Risk Assessment and Monitoring Plan

The notification C/ES/00/01 for maize NK603 under Directive 2001/18/EC is for import only, and thus there is no requirement for scientific information on environmental effects associated with the cultivation of maize NK603. In the environmental risk assessment the applicant has indicated that maize is highly domesticated and is not able to survive in the environment without cultivation. Maize plants are not winter hardy, have lost their ability to release seeds from the cob, and do not occur outside cultivated land in Europe, despite cultivation for many years. In addition there are no cross compatible wild relatives in Europe, and gene flow via pollen is largely restricted to neighbouring crops. Maize is a hybrid crop so that imported seeds will be a segregated F2 generation and not as fit as F1. Studies in Europe and elsewhere with NK603 have not shown any enhanced weediness or fitness. The environmental risk assessment concludes that the likelihood for unintended environmental effects due to the establishment and spread of NK603 maize will not to be different from that of traditionally bred maize. The GMO Panel agrees with this assessment.

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are to (1) 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) identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the environmental risk assessment. The scope of the monitoring plan provided by the applicant is in line with the intended uses for the GMO since the environmental risk assessment did not cover cultivation. The Panel advises that appropriate management systems should be in place to restrict seeds of maize NK603 entering cultivation, as the latter requires specific approval under Directive 2001/18/EC.

The Panel is not in a position to evaluate co-existence issues which relate to risk management and not risk assessment.

4. Food/Feed Safety Assessment

4.1 Toxicology

Safety of expressed novel proteins in maize NK603

The EPSPS enzyme occurs in a wide range of plants, fungi and certain micro-organisms and thus humans have a long history of dietary exposure to the protein. No adverse effects associated with its intake have been identified. Previous applications for glyphosate resistant crops containing the CP4 EPSPS protein have been evaluated and found to be safe for human and/or animal consumption (SCP, 1998a, 1998b; ACNFP, 1994). The GMO Panel is of the opinion that no scientific data have emerged which call for a change of this opinion.

The NK603 maize expresses the CP4 EPSPS and CP4 EPSPS L214P variant enzymes in addition to the natural maize EPSPS enzyme. Proteins derived from the *cp4 epsps* and *cp4 epsps L214p* genes were shown to be structurally and functionally equivalent. Evidence provided included (1)

modelling of CP4 EPSPS L214P protein structure (which showed that the amino acid substitution does not alter the predicted secondary and tertiary structure of the protein) (2) provision of evidence that the CP4 EPSPS protein domain containing the proline is highly variable in all known EPSPS proteins and (3) demonstration of equivalent enzyme activities for both CP4 EPSPS and CP4 EPSPS L214P proteins. Thus both proteins can be regarded as safe. Additional safety data were provided which included:

In vitro digestibility. Simulated gastric fluid (pH 1.2) containing pepsin and recombinant CP4 EPSPS or CP4 EPSPS L214P at 2.89:1 (w/w) ratio was incubated and analysed for intactness of the EPSPS proteins. More than 95-98% of the EPSPS protein was digested within 15 seconds. Intestinal digestion of CP4 EPSPS L214P was also simulated at a pancreatin: EPSPS ratio of 55:1 (w/w; buffer pH 7.5). Within four hours, more than 90% of the EPSPS was digested. These results confirm previous findings and show that the recombinant CP4 EPSPS enzymes were rapidly degraded in simulated gastric and intestinal fluid.

Lack of homology of both CP4 EPSPS proteins to known toxic proteins. The amino acid sequences of the CP4 EPSPS proteins were compared with the amino acid sequences of known toxic proteins using a bioinformatic approach based on computer algorithms. No relevant similarities between the sequence of the CP4 EPSPS proteins and sequences of toxic proteins were found.

Acute toxicity testing of both CP4 EPSPS proteins in mice. CP4 EPSPS and CP4 EPSPS L214P proteins produced in *Escherichia coli*, genetically modified to harbour the same *ctp2-cp4 epsps* cassettes as maize NK603, were fed at high single doses to CD-1 mice in acute gavage studies. Other mice received suitable control material. No adverse effects were observed in animals dosed with the CP4 EPSPS proteins. The LD50 of CP4 EPSPS and CP4 EPSPS L214P, therefore, exceeds the highest doses administered, i.e. 572 mg/kg body weight and 817 mg/kg body weight, respectively. The Panel does not advocate this type of study with proteins that have a known history of safe human exposure. No differences were detected between the EPSPS proteins purified from NK603 maize and the equivalent proteins expressed in *E. coli*. The expressed proteins were correctly processed at the amino terminus (cleavage of leader) and they were not glycosylated.

Safety of the whole GM food/feed

Sub-chronic (90-days) toxicity studies in rats fed maize NK603. No consistent differences in the measured clinical, biochemical and histological parameters were noted for rats fed on non-GM or NK603 maize, except for slightly elevated levels of average corpuscular volume and average corpuscular haemoglobin in female rats administered with a high dose. Since both parameters were calculated from other data (hematocrit/red blood cells and haemoglobin concentration/red blood cells, respectively), and no other observations of treatment related effects were made, the applicant suggests that an artifactual difference resulted from a slightly higher hematocrit or haemoglobin concentration and slightly lower red blood cell count at these sampling points. Furthermore, the applicant concludes that these findings are of no biological significance. The Panel accepts this as a reasonable interpretation of the data. The Panel also found the doses chosen for the study (11% or 33% of diet) appropriate, as they did not distort the nutritional balance of the experimental animals. The standard rodent diet used by the test laboratory contains approximately 33% maize grain.

4.2 Allergenicity

Some Member States raised questions about the suitability of approaches used for allergenicity testing. These strategies concentrate on characterisation of the source of the recombinant

protein, the potential of the newly expressed protein to induce *de novo* sensitisation, or to elicit allergic reactions in already sensitised persons, and whether the transformation may have altered the allergenic properties of the modified food. A weight of evidence approach is recommended, taking all information obtained with various test methods into account, since no single experimental method yields decisive evidence for allergenicity (EC, 2003; Codex Alimentarius Commission, 2003).

An allergy risk evaluation of CP4 EPSPS protein has been completed for previous applications evaluated by the EC Scientific Committees and the national competent authorities using the guidelines proposed by Metcalfe et al., 1996. These included absence of known allergenicity of the source, absence of sequence homology with known allergens and rapid and extensive degradation by proteolytic enzymes. The Panel is not aware of any new information on allergenicity which requires a change of this opinion. Nor is the Panel aware of any new tests which produce more relevant or accurate information on possible allergenicity of the protein and which provide a higher guarantee of safety.

Member States also raised the question of the possibility that new allergens might arise. The applicant has provided a bioinformatic analysis of potential allergenicity, as well as toxicity and pharmacological activity, of putative peptides encoded at the 3' and 5' junctions of the NK603 event and plant genomic DNA. Sequences spanning the junction were translated from stop codon to stop codon in all frames and each frame compared to appropriate sequence databases including those for allergens and toxins. Data provided demonstrate that in the unlikely event that junction polypeptides were translated they would not share a sufficient degree of sequence similarity or identity to known allergens or toxins.

Another related issue is that allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the host e.g through qualitative or quantitative modifications of the pattern of expression of endogenous proteins. This issue does not appear relevant to the Panel since maize is not considered a major allergenic food and possible overexpression of any endogenous protein which is not known to be allergenic would be unlikely to alter the overall allergenicity of the whole plant.

4.3 Nutritional Assessment of GM food/feed

Feeding studies on broilers. A nutritional performance study with suitable dosages of maize NK603 grain and non-GM maize grain was carried out with rapidly growing broilers, which reach full size within approximately six weeks. The applicant states that the NK603 maize grain used in the 90-day toxicity study in rats and the 42-day broiler feeding study was obtained from plots treated with glyphosate herbicide. The maize grain used in the studies was analysed for residual herbicide content. The level of glyphosate in the NK603 grain was below the maximum residue level, as regulated under EU legislation on plant protection products.

The only statistically significant difference occurred between fat pad weights of broilers fed maize NK603 and broilers fed non-GM maize. The weights were 0.034 kg and 0.037 kg (1.5 % and 1.7 % of body weight), respectively. The difference is not considered to be of biological significance. The values are within the variability (0.024-0.063 kg) reported in the literature (Esteve-Garcia and Llaurodo, 1997; Kidd and Kerr, 1997; Lei and Van Beek, 1997; Smith et al., 1998; Farran et al., 2000; Peak et al., 2000). The Panel also wishes to point out the published data on feeding studies with maize NK603 and non-GM maize on Angus-continental cross steers and Holstein dairy cows (Erickson et al., 2003; Grant et al., 2003; Ipharraguerre et al., 2003). The data show that the nutritional value of NK603 is equivalent to its non-GM comparator.

4.4 Residues and metabolites of the herbicide

A Member State has raised the issue of the possible occurrence of residues of glyphosate and its metabolite (AMPA) in maize NK603, and the effects of these compounds on animal and human health. The Panel recognizes the importance of the issue and notes that the risk assessment of such compounds is within the scope of Directive 91/414/EEC⁴ (EC, 1991).

CONCLUSIONS

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission has requested the European Food Safety Authority to issue two scientific opinions as to whether the placing on the market of NK603 maize and derived food and feed products, for import and processing, is likely to cause any adverse effects on human health and the environment in the context of Regulation (EC) No 258/97⁵ and Directive 2001/18/EC. The EFSA GMO Panel considered the information made available by the applicant as sufficient to evaluate the safety of NK603 maize and derived products, food and feed ingredients and to address all the specific questions raised by the Member States related to the risk assessment. Therefore additional experimental studies are not deemed necessary.

Maize NK603 has been developed for tolerance to glyphosate herbicide by the introduction of a glyphosate tolerant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene from *Agrobacterium* sp. The Panel has considered information provided on (1) the molecular inserts within the transgenic event, (2) the chemical composition of the GM and non-GM maize, (3) the safety of the proteins expressed and (4) the potential for risks associated with any changes to the toxicological, allergenic and nutritional properties of NK603. Having considered the evidence, the GMO Panel is of the opinion that NK603 maize is as safe as conventional maize and therefore the placing on the market of NK603 maize for food or feed or processing is unlikely to have an adverse effect on human and animal health and, in that context, the environment.

DOCUMENTATION PROVIDED TO EFSA

With regard to the application under Directive 2001/18/EC:

1. Letter, dated 7 August 2003 with ref. SANCO.D. MW/dj D(2003) 450049, from Mrs Jaana Husu Kallio from the Health & Consumer Protection Directorate-General requesting a consultation of the Scientific Panel on Genetically Modified Organisms and following correspondence with EFSA (letter, dated 13 August 2003 with ref. MCM/D (2003) – 00255, from Mr. Geoffrey Podger and letters, dated 18 and 23 September 2003 with ref. SANCO.D.5 MW/ D(2003) 4500091 and SANCO.D.5 MW/ D(2003) 4500094, from Mrs Jaana Husu Kallio)

⁴ Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market.

⁵ Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients.

2. A letter from Monsanto to the competent authority of Spain concerning submission of the notification.
3. The report of the assessment of the notification carried out by the competent authority of Spain (Ministerio de Medio Ambiente).
4. The summary of the notification.
5. Roundup Ready maize line NK 603 – Application for consent to place on the market a genetically modified higher plant under Directive 2001/8/EC (Monsanto).
6. Additional information submitted by Monsanto in response to comments and objections raised by the competent authorities of Member States.
7. The objections maintained by Member States

With regard to the application under Regulation 258/97:

1. Letter, dated 24 July 2003 with ref. SANCO AN/dlc-D(03)440360, from Mrs Paola Testori Coggi from the Health & Consumer Protection Directorate-General requesting a consultation of the Scientific Panel on Genetically Modified Organisms and following correspondence with EFSA (letter, dated 5 August 2003 with ref. MCM/D (2003) – 00242, from Mr. Geoffrey Podger and letter, dated 27 August 2003 with ref. PD/ko/D440449 (2003), from Mrs. Paola Testori Coggi).
2. Roundup Ready maize line NK 603 – Application for approval under Regulation (EC) No. 258/97 concerning Novel Foods and Food Ingredients (Monsanto).
3. Herbicide-tolerant maize (NK603) Assessment of safety for the consumer, in accordance with European Regulation 258/97 concerning novel foods and novel food ingredients (Gezondheidsraad, NL).
4. Member States' comments/objections.
5. Reaction by Monsanto to the Member States' comments/objections.

REFERENCES

- ACNFP, (1994) Annual Report 1994, Appendix 4, Advisory Committee on Novel Foods and Processes, London, UK.
http://www.foodstandards.gov.uk/multimedia/webpage/acnfp_report_1994
- ACRE, 2002. Advisory Committee on Releases of GM crops into the Environment. ACRE's response to concerns raised in written representations and submissions associated with the CHARDON LL public hearing and to statements made at ACRE's open hearing relating to the safety assessment of T25 GM maize conducted under Directive 90/220/EEC.
<http://www.defra.gov.uk/environment/acre/advice/advice20c.htm>
- Codex Alimentarius Commission, 2003. Codex principles and guidelines on foods derived from biotechnology Foods Derived from Biotechnology. Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation: Rome.
<ftp://ftp.fao.org/codex/standard/en/CodexTextsBiotechFoods.pdf>
- EC, 1991. Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Official Journal of the European Communities L230, 1-32.

- http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31991L0414&model=guichett
- EC, 1997. Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Official Journal of the European Communities L43, 1-7.
http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31997R0258&model=guichett
- EC, 2001. 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. Official Journal of the European Communities L106, 1-39.
http://europa.eu.int/eur-lex/pri/en/oj/dat/2001/l_106/l_10620010417en00010038.pdf
- EC, 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities L31, 1.2.2002, p. 1-24.
http://europa.eu.int/eur-lex/pri/en/oj/dat/2002/l_031/l_03120020201en00010024.pdf
- EC, 2003. Guidance document for the risk assessment of genetically modified plants and derived food and feed, prepared by the Joint Working Group on Novel Foods and GMOs, 6-7 March 2003. http://europa.eu.int/comm/food/fs/sc/ssc/out327_en.pdf
- Erickson, G.E., Robins, N.D., Simon, J.J., Berger, L.L., Klopfenstein, T.J., Stanisiewski, E.P. and Hartnell, G.F., 2003. Effect of feeding glyphosate-tolerant (Roundup –Ready events GA21 or nk603) corn compared with reference hybrids on feedlot steer performance and carcass characteristics. *J. Anim. Sci.*, 81, 2600-2608.
- Esteve-Garcia, E. and Llauro, L., 1997. Performance, breast meat yield, and abdominal fat deposition of male broiler chickens fed diets supplemented with DL-methionine or DL-methionine hydroxy analogue free acid. *Br. Poult. Sci.*, 38, 397-404.
- Farran, M.T., Khalil, R.F., Uwayjan, M.G. and Ashkarian, V.M., 2000. Performance and carcass quality of commercial broiler strains. *J. Appl. Poult. Res.*, 9, 252-257.
- Grant, R.J., Fanning, K.C., Kleinschmit, D., Stanisiewski, E.P. and Hartnell, G.F., 2003. Influence of glyphosate-tolerant (event nk603) and corn rootworm protected (event MON863) corn silage and grain on feed consumption and milk production in Holstein cattle. *J. Dairy Sci.*, 86, 1707-1715.
- Hull, R., Covey, S.N, Dale P.J., 2000. Genetically modified plants and the 35S promoter: assessing the risks and enhancing the debate. *Microbial Ecology in Health and Disease*, 12, 1-5.
- Huang, C.Y, Ayliffe M.A., Timmis, J.N., 2003. Direct measurement of the transfer rate of chloroplast DNA into the nucleus. *Nature*, 422, 72-76
- Ho, M. W., Ryan, A., Cummins, J. (1999). Cauliflower mosaic viral promoter - a recipe for disaster. *Microbial Ecology in Health and Disease*, 11, 194-197.
- Ipharraguerre, I.R., Younker, R.S., Clark, J.H., Stanisiewski, E.P. and Hartnell, G.F., 2003. Performance of lactating dairy cows fed corn as whole plant silage and grain produced from a glyphosate-tolerant hybrid (event NK603). *J. Dairy Sci.*, 86, 1734-1741.
- Jones, L., 2002. Revealing micro-RNAs in plants. *Trends in Plant Science*, 7, 473-475.

- Kidd, M.T. and Kerr, B.J., 1997. Threonine responses in commercial broilers at 30 to 42 days. *J. Appl. Poult. Res.*, 6, 362-367.
- Lei, S. and Van Beek, G., 1997. Influence of activity and dietary energy on broiler performance, carcass yield and sensory quality. *Br. Poult. Sci.*, 38, 183-189.
- Metcalfe D.D., Astwood J.D., Townsend R, Sampson HA., Taylor SL., Fuchs RL., 1996. Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Crit. Rev. Food. Sci. Nutr.* ,36(S), 165-186.
- Peak, S.D., Walsh, T.J., Benton, C.E. and Brake, J., 2000. Effects of two planes of nutrition on performance and uniformity of four strains of broiler chicks. *J. Appl. Poult. Res.*, 9, 185-194.
- SCP, 1998a. Opinion of the Scientific Committee on Plants regarding the genetically modified cotton, tolerant to glyphosate herbicide notified by the Monsanto Company (notification C/ES/97/01).
http://europa.eu.int/comm/food/fs/sc/scp/out17_en.html
- SCP, 1998b. Opinion of Scientific Committee on Plants regarding submission for placing on the market of fodder beet tolerant to glyphosate notified by DLF-Trifolium, Monsanto and Danisco Seed (notification C/DK/97/01).
http://europa.eu.int/comm/food/fs/sc/scp/out16_en.html
- Smith, E.R., Pesti, G.M., Kakalli, R.I., Ware, G.O. and Menten, J.F.M., 1998. Further experiments on the influence of genotype and dietary protein on the performance of broilers. *Poult. Sci.*, 77, 1678-1687.

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