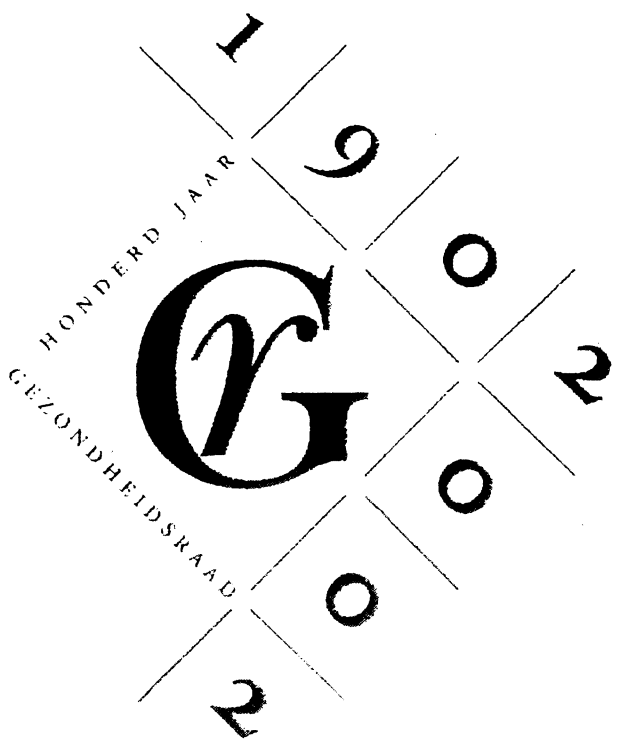
Herbicide-tolerante maïs (NK603)

Herbicide-tolerant maize (NK603)

Commissie Veiligheidsbeoordeling nieuwe voedingsmiddelen
Committee on the Safety Assessment of Novel Foods



Gezondheidsraad

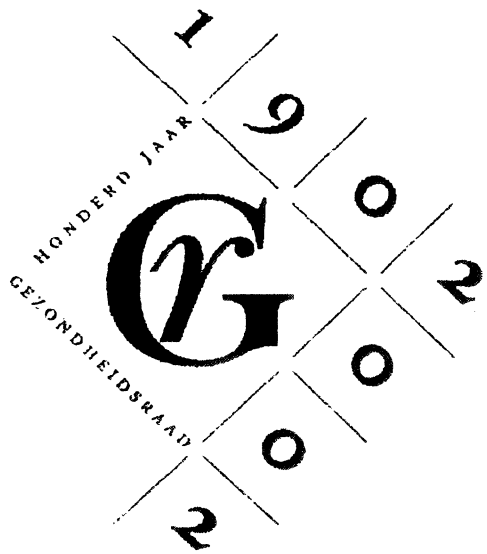
2002/04VNV

Herbicide-tolerante maïs (NK603)

Beoordeling van de veiligheid voor de consument, volgens de Europese verordening 258/97 betreffende nieuwe voedingsmiddelen en nieuwe voedselingrediënten (vertrouwelijke versie)

Herbicide-tolerant maize (NK603)

Assessment of safety for the consumer, in accordance with European Regulation 258/97 concerning novel foods and novel food ingredients (confidential version)



Herbicide-tolerant maize (NK603)

Assessment of safety for the consumer, in accordance with European Regulation 258/97 concerning novel foods and novel food ingredients (confidential version)

Letter to the Dutch Minister of Health, Welfare and Sport

On August 13, 2002, professor dr JGAJ Hautvast, Vice-President of the Health Council of the Netherlands wrote as follows to the Minister of Health, Welfare and Sport:

Herewith I present you an advisory report that is prepared in response to your predecessor's request for advice regarding the safety of novel foods and novel food ingredients, also made on behalf of the Minister of Agriculture, Nature Management and Fisheries. This advice is a so called initial assessment in the context of European Regulation (EC) 258/97, concerning Herbicide-tolerant maize (NK603). The assessment was carried out by the Committee on the Safety Assessment of Novel Foods of the Health Council of the Netherlands.

This advisory report is also presented to the Minister of Agriculture, Nature Management and Fisheries.

Signed
professor dr JGAJ Hautvast

Herbicide-tolerant maize (NK603)

Assessment of safety for the consumer, in accordance with European Regulation 258/97 concerning novel foods and novel food ingredients (confidential version)

Health Council of the Netherlands:
Committee on the Safety Assessment of Novel Foods

to:

the Minister of Health, Welfare and Sport

the Minister of Agriculture, Nature Management and Fisheries

No. 2002/04VNV, The Hague, August 13, 2002

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect tot public health issues" (Section 21, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport; Housing, Spatial Planning & the Environment; Social Affairs & Employment, and Agriculture, Nature management & Fisheries.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

Preferred citation:
Health Council of the Netherlands: Committee on the Safety Assessment of Novel Foods. Herbicide-tolerant maize (NK603). The Hague: Health Council of the Netherlands, 2002; publication no. 2002/04VNV.

Contents

Executive summary, conclusions and recommendations

The applicant, Monsanto Company, presented a safety dossier for the genetically modified maize line NK603. This dossier contains molecular biological, nutritional and toxicological information. The reference is a conventional maize line with a history of safe use within the European Union.

The modified maize line differs from a conventional line, due to the presence of the *cp4 epsps* gene and its expression product, the CP4 EPSPS protein. The inserted gene is of bacterial origin and renders the maize plant tolerant to the herbicide glyphosate. Two consecutive copies of this gene are present at a single location in the DNA of NK603 maize.

Molecular biological analysis of the new crop revealed a slight difference between these two copies. As a result of this, not only is the intended CP4 EPSPS protein produced, but a variant of this (CP4 EPSPS L214P) can also be produced, containing one different amino acid. There are no indications that these new proteins are toxic or allergenic for humans in the concentrations at which they occur.

The intended change is associated with an unintended rearrangement of the DNA at the edge of the insertion, which has also been analysed in detail. There are no indications that the changes in the maize plant genome result in the unintended production of other new proteins.

The modified maize line's composition has been compared with that of the near isogenic, non-modified parent line by means of a chemical analysis of a large number of components: micronutrients, macronutrients, antinutrients and secondary metabolites. To this end, field trials were conducted at several locations, the results of

which were mostly processed for each location separately. Observed variations in the components investigated remained within the figures cited in the references and have no health-related consequences.

Furthermore, in a subchronic toxicity test on animals, no adverse effects of the modification in NK603 maize were observed.

The Committee is of the opinion that the information in the dossier provides sufficient basis for a safety evaluation. The dossier contains a correct interpretation of the data submitted. Based on current scientific knowledge, the Committee's opinion is that the consumption of NK603 maize and foods and food ingredients derived from this is just as safe for humans as the consumption of non-genetically modified maize and maize products.

Chapter 1

Introduction

On 29 May 2001 the Minister of Health, Welfare and Sport requested the advice of the Committee on the Safety Assessment of Novel Foods (VNV), hereinafter referred to as 'the Committee', concerning the safety for consumers of foods and food ingredients produced from a new maize variant. This maize, designated NK603, originates from a plant (*Zea mays* L.) which, as a result of genetic modification, produces a modified biosynthesis enzyme that renders it tolerant to the herbicide glyphosate. The glyphosate tolerance of maize line NK603 makes it possible to control weeds in the field during growth of the maize plants by glyphosate treatment, without damaging the maize plants. The maize variant has been developed by Monsanto, who also submitted the request (Mon01). The request contains a safety evaluation with a number of accompanying research reports.

In June 2001, the Committee requested the applicant to complete the dossier by sending all of the data cited in the references. The applicant supplied these data in July 2001. In December 2001, the Committee requested additional data about the molecular characterisation of the NK603 maize and the design of the field trials conducted. The Committee also requested the applicant to submit the results of additional animal experiments. In March 2002, the applicant supplied the new information requested (Mon02).

The Committee devoted several meetings to discussing the dossier and completed its assessment in August 2002. This advisory report contains the Committee's findings.

Chapter 2

Completeness and accuracy of the dossier

2.1 Administrative data

The name and address of the applicant, hereafter referred to as 'Monsanto' or 'the applicant', are as follows:

Monsanto Company, represented by Monsanto Europe S.A., 270-272 Avenue de Tervuren, 1150 Brussels, Belgium.

2.2 General description of the food

The application concerns the marketing and trading of NK603 maize on the European market for further processing into foods and food ingredients. This application by Monsanto to the Dutch competent authority has two components. Firstly, an authorisation is requested in accordance with article 3, subsection 2 of EC Regulation 258/97 for all foods and food ingredients derived from the maize (EC97). Secondly, the Committee's opinion is requested concerning the substantial equivalence of maize oil and hydrolysates from maize starch, obtained from NK603 maize with the same food ingredients produced from conventional maize. The Committee chose to assess the safety of the genetically modified maize kernels for consumption and believes this assessment is also applicable to the products obtained from further processing of these maize kernels.

2.3 Classification of the food for assessment

The file contains arguments for classification in class 3.1, one of the six main classes and sub classes of novel foods as referred to in table 1, section 1 of Recommendation 97/618 of the European Commission (EC97a). This concerns a genetically modified plant, the conventional variant of which has a history of safe use in the European Union. The Committee concurs with this classification.

2.4 Information about the food

The applicant structured the information that is essential for a safety assessment of novel food consumption in accordance with the themes prescribed in Recommendation 97/618 of the European Commission (EC97a):

I Specification of the novel food (NF)

II Effects of the production process applied to the NF

III History of the organism used as the source of the NF

IV Effect of the genetic modification on the properties of the host organism

V Genetic stability of genetically modified organism (GMO) used as NF source

VI Specificity of expression of novel genetic material

VII Transfer of genetic material from the GMO

IX Anticipated intake and extent of use of the NF

X Information from previous human exposure to the NF or its source

XI Nutritional information on the NF

XII Microbiological information on the NF

XIII Toxicological information on the NF

For every subject, the applicant follows each step in the flow charts and refers to the appendices or the references for the data used.

2.5 Brief summary by the applicant

The file contains a brief summary that was sent to the EU member states in accordance with article 6, section 2 of the European Regulation (EC) 258/97 (EC97).

2.6 Other assessments

Monsanto has also notified the Ministry of Agriculture, Nature Management and Fisheries about this dossier, within the scope of the voluntary check on the safety of animal feed. The National Institute for Quality Control of Agricultural Products is conducting the animal feed assessment. Permission to treat this maize in the field with the herbicide glyphosate rests with the Board for the Authorisation of Pesticides, which also establishes a residue tolerance for foods derived from the maize.

2.7 Proposal for labelling by the applicant

The dossier contains a labelling proposal, which incorrectly states maize line GA21 instead of NK603 in several places. Labelling should satisfy the requirements in EC Regulations 258/97 (EC97), 1139/98 (EC98), and 49/2000 (EC00). However, in the Netherlands, a labelling proposal is discussed in the Regular Consumer Goods Act Consultations and is not further assessed by this Committee.

Chapter 3

Interpretation and evaluation of the data presented

3.1 I Specification of the novel food (NF)

This application concerns a maize line in which two copies of the *cp4 epsps* gene have been introduced, which originate from the soil bacterium *Agrobacterium* sp., strain CP4. This gene codes for a variant of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS). Another form of EPSPS occurs naturally in plants, which is inactivated when the herbicide glyphosate is applied. This results in disruption of the biosynthesis of aromatic amino acids after a plant has been treated with glyphosate. The CP4 EPSPS has less affinity for glyphosate. Therefore, the transgenic maize plant is less sensitive to this herbicide. It is the opinion of the Committee that the data presented in this dossier are representative of products that are marketed under the name 'Roundup Ready maize line NK603'.

3.2 II Effects of the production process applied to the NF

Whole maize kernels are used as animal feed on a large scale, but only on a small scale for direct human consumption (in the case of sweet corn). However, flour, starch and oil obtained from maize are important base materials in the production of foods. For a large proportion of the maize starch, this involves conversion into syrups or ethanol.

The modification in maize line NK603 is of agronomic importance and does not influence the production processes used to process maize into foods and food ingredients. The applicant describes these production processes in detail.

3.3 III History of the organism used as the source of the NF

The source of the novel food plant is a conventionally cultivated maize variety, *Zea mays* L., line AW x CW. A DNA fragment containing two copies of the *cp4 epsps* gene with different regulation signals has been added to the genome of this maize. The applicant provides an overview of the worldwide production of maize and the production, import and consumption of this crop in the EU.

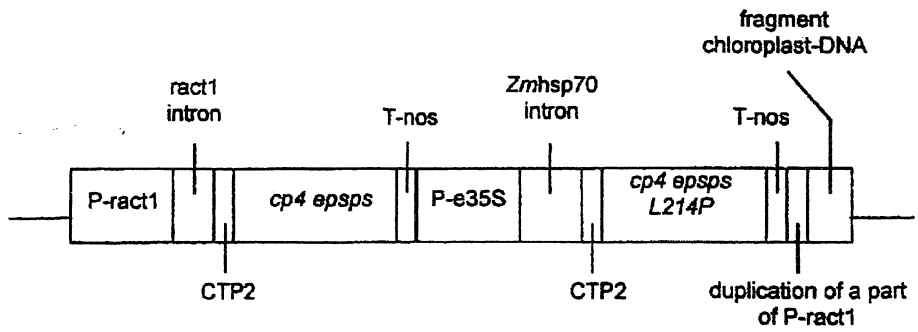
3.4 IV Effect of the genetic modification on the properties of the host organism

This request concerns a maize line in which two copies of the *cp4 epsps* gene have been introduced, due to the transfer of a single, linear DNA fragment. This was accomplished by using a particle acceleration method to transfer the novel DNA, precipitated onto microscopic gold particles, into maize cells. The transgenic NK603 plant was regenerated from a single maize cell after incorporation of the novel DNA fragment into its chromosomal DNA. During this process, glyphosate was used to select transformed cells. In the transgenic plant, two bases have been changed in one of the two copies of the *cp4 epsps* gene and consequently the two gene products differ by one amino acid. In addition to the intended modification, two extra DNA fragments were unintentionally incorporated at the location of the insertion.

The *cp4 epsps gene* originates from the bacterium *Agrobacterium* sp. strain CP4 and codes for the protein CP4 EPSPS. EPSPS is the standard abbreviation for 5-enolpyruvylshikimate-3-phosphate synthase, an enzyme important for the biosynthesis of aromatic amino acids. It is found in plants, bacteria and fungi. The introduced CP4 EPSPS protein catalyses the same reaction as the EPSPS protein, which naturally occurs in maize, but has far less affinity for the herbicide glyphosate. Therefore, when treated with glyphosate, the NK603 maize suffers less damage than the weeds because its metabolism is scarcely disrupted.

Both copies of the *cp4 epsps* gene are preceded by a sequence from the plant *Arabidopsis thaliana* (CTP2), which is translated into a peptide that ensures the transport of CP4 EPSPS to the chloroplasts, where EPSPS is also naturally localised. In both copies the coding sequence is followed by the nopaline synthase terminator sequence from the bacterium *Agrobacterium tumefaciens* (T-nos). One copy of the *cp4 epsps* gene is preceded by the promoter and intron sequence of the rice actin gene (Pract1 and ract1 intron). The other copy is under the control of the 35S promoter from cauliflower mosaic virus, in which the so-called enhancer region is duplicated for

Schematic representation of the modification in the DNA of NK603 maize (terms used are clarified in the text)



optimal expression (P-e35S). This promoter is followed by the *Zm*hsp70 intron from maize.

The modification in maize line NK603 was analysed using Southern blotting. In addition, flanking DNA sequences were determined by analysis of PCR products. The NK603 maize was found to contain one complete copy of the DNA fragment described above. The Committee asked the applicant for an experimental confirmation of the expected nucleotide sequence of the insertion in the DNA of the transgenic plant. Research conducted to this end showed that the second copy of the *cp4 epsps* gene differed from the DNA used for the transformation by two bases. This results in one alternative amino acid being incorporated into the protein formed, which is referred to as CP4 EPSPS L214P. This change does not affect the protein's enzymatic activity.

Immediately adjacent to the intended insertion, two additional DNA fragments are present as an unintended effect of the modification. The first extra fragment is a duplication of 217 base pairs of the intended insertion, linked in inverse orientation. It consists of 50 base pairs of polylinker sequence and the first 167 base pairs of the enhancer region of the rice actin promoter. The second extra fragment consists of 305 base pairs, homologous to chloroplast DNA. The applicant states that the presence of these two extra fragments could be due to co-integration during the transformation process.

This rearrangement of the DNA downstream from the intended insertion was analysed in detail. No detectable transcription of the 217 base pair fragment was observed in Northern blot analysis. However, RT-PCR experiments presented later indicated the formation of mRNA containing this sequence. The formation of proteins other than CP4 EPSPS and CP4 EPSPS L214P as a result of this, however, is most

unlikely. The sequence of the fragment of chloroplast DNA found or from the fusion locations upstream and downstream of the insertion could theoretically be translated into additional proteins. Although actual formation of these proteins is unlikely, the applicant has determined that none of the putative protein products expresses similarities with known allergenic or toxic proteins. Flanking sequences from the maize genomic DNA, 307 base pairs long upstream and 497 base pairs long downstream of the modification, were determined by analysing PCR products. The site of the insertion was also analysed in the DNA of the non-transgenic parent plant. This revealed that three base pairs were removed from the maize genome when the new DNA was inserted in maize line NK603. As a purified fragment of the original plasmid was used for the transformation, the *ori*-sequence and the *nptII* antibiotic resistance gene from this plasmid are not present in the DNA of maize line NK603. Southern blotting confirmed this.

The Committee is of the opinion that with this, a thorough analysis of the genetic modification has been conducted. No indication was found of other effects of the genetic modification than the production of proteins CP4 EPSPS and CP4 EPSPS L214P.

3.5 V Genetic stability of the genetically modified organism (GMO) used as NF source

The inheritance of glyphosate tolerance in the progeny of the original transformant was studied in six generations of backcrossing with the maize line B73. In only one of the six cases was the result significantly different from the expected 1:1 segregation of this characteristic. As a possible explanation, the applicant states that the selection pressure sometimes also causes a selection of the gametes (Sar94, Tou95). The glyphosate tolerance was studied in three additional generations of progeny, created by self-fertilisation of heterozygous glyphosate tolerant plants. In these cases no significant differences were found from the expected 1:2:1 distribution for the homozygous resistant, heterozygous resistant and homozygous sensitive plants respectively.

The DNA of the plants from the first and fifth generation of the backcrosses were analysed for the presence of the *cp4 epsps* gene using Southern blotting. The same hybridising fragments were found in both of the generations tested.

The Committee concludes that the inserted DNA was inherited in a stable manner and expressed in the generations investigated.

3.6 VI Specificity of expression of novel genetic material

The applicant describes the expression of the CP4 EPSPS protein in maize kernels and forage from field experiments, carried out at eight different locations in the United States. In all cases, the CP4 EPSPS protein could be detected by means of an ELISA technique with a specific antiserum. Furthermore, the CP4 EPSPS formed in the maize line NK603 was compared, using Western blotting, with CP4 EPSPS from transgenic soya (A5403) and with CP4 EPSPS that was produced in *E. coli*. The protein in these three preparations had a similar apparent molecular weight and a similar reactivity with the antiserum used.

3.7 VII Transfer of genetic material from the GMO

The applicant describes the various known mechanisms for the horizontal gene transfer and points out that the DNA inserted in the NK603 maize does not contain specific sequences which would promote the transfer or mobilisation of a part of this DNA. The applicant also notes that the possibility of DNA transfer from the novel food to resident microflora in the human digestive system is the most likely mechanism that could be important for the food safety evaluation at this point. On the basis of data taken from the references, the applicant reaches the conclusion that the occurrence of horizontal gene transfer from NK603 maize is highly unlikely and that the nature of the genetic modification in this maize is such that a possible transfer to bacteria in the human digestive system would not lead to a selective advantage for these bacteria.

The Committee notes that we consume large quantities of plant and animal DNA on a daily basis and that it is conceivable that parts of this DNA reach the intestines in the form of intact gene fragments. There they could also be transferred to the resident microflora or to human cells. Should this occur, there will be little, if any, expression of these genes in practice, as they are not linked to a suitable promoter. If these genes were nevertheless to be expressed in micro-organisms, they would, in the vast majority of cases, not provide the bacteria concerned with a competitive advantage and the host would not be disadvantaged. This is particularly the case for the CP4 EPSPS gene.

Therefore, the Committee shares the applicant's viewpoint that no adverse effects are to be expected resulting from transfer of genetic material from NK603 maize, if such transfer would occur.

3.8 IX Anticipated intake and extent of use of the NF

Although the maize plant and whole maize kernels are mostly used as animal feed, maize oil and maize starch derived from the maize kernels are used in large quantities in products for human consumption. Syrups and ethanol produced from maize starch are also used on a large scale in the food industry.

The glyphosate tolerance of maize line NK603 is of agronomic importance and could be used worldwide. Although NK603 maize will therefore initially just replace other maize, it cannot be ruled out that the cultivation of the new maize line could lead to a better competitive position owing to a more efficient production of the crop concerned. In that case, the human consumption of products derived from maize (in particular oil and starch) could increase compared to equivalent ingredients derived from other crops. There are no objections to this from a nutritional viewpoint.

3.9 X Information from previous human exposure to the NF or its source

Conventional maize is cultivated on an almost worldwide basis and has a long history of safe use.

3.10 XI Nutritional information on the NF

The applicant describes the results of the compositional analysis of plant material, derived from field trials carried out in the United States in 1998 (two replicated trials in Ohio and Illinois and six non-replicated trials in Iowa, Illinois, Indiana and Kansas) and in Europe in 1999 (two replicated trials in Southern France and Italy and two non-replicated trials in Northern France). Data from replicated trials were statistically analysed per location and the results from all field trials in the United States were combined into an extra set of data for processing. In all cases, non-transgenic maize was compared with maize line NK603, which was treated with glyphosate.

The formation of new metabolites from the herbicide can be ruled out, due to the mechanism of action of the glyphosate resistance via the CP4 EPSPS enzyme. The moisture level, ash, carbohydrate, protein, fat and fibre (ADF and NDF) were determined in maize kernels and forage. The level of calcium, copper, iron, magnesium, manganese, phosphorous, potassium and zinc in the maize kernels was also determined. Furthermore, the amino acid and fatty acid composition was determined as well as the level of phytic acid, trypsin inhibitor and vitamin E. Statistically significant differences were considered with respect to the total outcomes

for different locations and with respect to known values in the references. The differences found were within the natural variation for the components concerned.

Maize kernels from field trials in the United States were analysed for the secondary metabolites ferulic acid, *p*-coumaric acid, raffinose and furfural. Three sets of data were used for this, namely, the separate sets of results from the two replicated trials in Illinois and Ohio and a combined data set taken from several field trials. No statistically significant differences were found between maize kernels from NK603 and the non-transgenic control. Furfural could not be detected in any of the samples analysed. In the comparison between NK603 maize and non-transgenic maize, the applicant also describes an animal feeding experiment with broiler chicks, where a number of parameters regarding the growth of the chicks were investigated. In as far as significant differences were found, these remained within the known values cited in the references.

The Committee endorses the applicant's conclusion that NK603 maize does not differ nutritionally from conventional maize.

3.11 XII Microbiological information on the NF

In view of the nature of the modification in the maize line NK603, there is no reason to expect that changes in the presence of micro-organisms or microbial metabolites will occur.

3.12 XIII Toxicological information on the NF

The applicant argues that the CP4 EPSPS protein is substantially similar to other EPSPS proteins, present in our food as natural components of plants, fungi and bacteria and for which no adverse effects are known.

The possible acute oral toxicity of the CP4 EPSPS protein has been investigated in mice. In this study, no adverse effects were observed for a maximum tested dose of 572 mg of the protein per kg of body weight.

The stability of CP4 EPSPS protein *in vitro* was investigated using simulated gastric and intestinal fluids. In this digestion test, the half-life of the protein was less than 15 seconds in simulated gastric fluid and less than 10 minutes in simulated intestinal fluid. CP4 EPSPS L214P was also rapidly digested in simulated gastric fluid.

Furthermore, the amino acid sequences of CP4 EPSPS and CP4 EPSPS L214P were compared with the amino acid sequences of known toxic proteins. This revealed no similarities. Comparisons with amino acid sequences from other known proteins only revealed similarities with other EPSPS proteins. A comparison of the amino acid

sequences of CP4 EPSPS and CP4 EPSPS L214P with known allergens revealed no similarities. In this comparison, sequences were sought containing at least eight contiguous identical amino acids.

A characteristic of known food allergens is the high concentration at which they are present in the food concerned. However, this is not the case for NK603 maize, where the CP4 EPSPS protein forms approximately 0.01% of the total protein content of the maize kernel. Furthermore, known food allergens are, in general, fairly resistant to digestive breakdown, whereas this is unlikely for CP4 EPSPS and CP4 EPSPS L214P in view of the results from the *in vitro* digestion experiments.

The results of the investigations described are supported by the fact that glyphosate resistant soya, which also contains the CP4 EPSPS protein, is already cultivated and consumed on a large scale and yet no adverse effects have been reported.

The Committee is of the opinion that, in so far as the proteins CP4 EPSPS and CP4 EPSPS L214P are present in the novel food or food ingredients derived from this, there is no cause to expect any toxicity or allergenicity.

As the molecular biological analysis cannot rule out the fact that other unintended changes can be present in the genome of the plant, the Committee proposed that an extra guarantee of safety must be obtained for the long-term human consumption of NK603 maize. At the Committee's request, the applicant submitted the results of a subchronic toxicity test, conducted with Sprague Dawley albino rats. The test groups received 33% or 11% w/w NK603 maize in their feed over a 90-day period. Control groups received 33% or 11% w/w of comparable, non-modified maize in their feed. In the 11% groups, the feed was supplemented with 22% w/w comparable non-modified maize. In addition to this, maize kernels from six other commercial maize varieties were administered to control groups (33% w/w). This set of six control groups were used as a reference for the NK603 test groups, in addition to the comparison of the test groups with the direct control groups.

All of the test and control groups contained 20 male and 20 female rats. The observations consisted of clinical observations (daily on visible symptoms of toxicity and weekly by means of a general examination) and measurements of growth and food uptake (weekly). Halfway through the study and at the end of it, blood and urine were taken from 10 rats out of each group of 20. Urine samples were analysed for general as well as chemical and biochemical parameters. Several haematological and clinical chemistry parameters were determined for the blood. However, coagulation factors were only investigated at the end of the study.

Once the study had been completed, the organs from of all of the animals were examined macroscopically. The weight of a number of organs was also determined (adrenals, brain, heart, kidneys, liver, ovaries, spleen and testes). A number of organs

from the animals in the high dose test group NK603 and the high dose control group for comparable non-modified maize were also investigated microscopically (adrenals, brain, colon, duodenum, heart, ileum, jejunum, kidneys, liver, mesenteric lymph node, ovaries, pancreas, rectum, spleen, stomach, testes and thyroid/parathyroid).

Any significant differences found were not attributable to the administration of NK603 maize. In the Committee's opinion, the results of the subchronic toxicity study in rats carried out by the applicant show that no adverse effects from the consumption of NK603 maize can be expected as a consequence of possible unknown changes in the genome of the plant.

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A Request for advice

B The committee

C EU-procedure

D Executive summary of the dossier

Annexes

Annex **A**

Request for advice

On 18 August 1999, the Minister of Health, Welfare and Sport wrote as follows to the President of the Health Council of the Netherlands (under reference GZB/VVB 993428):

Since May 1997, Regulation (EC) 258/97 concerning novel foods and novel food ingredients has been in force in the European Union. Under the Regulation, the safety of novel foods has to be assessed as part of a community procedure.

Following discussions regarding the possibility of the Health Council making such assessments, the State Secretary for Agriculture, Nature Management and Fisheries and I wish the Council to take responsibility for safety assessment for a period of several years during the fist phase of implementation of European Regulation (EC) 258/97. It is considered appropriate that the Health Council should initially take on this role because the assessment activities will be of an experimental nature, involving both a new form of assessment (i.e. pre-marketing assessment) and, in many cases, new categories of foodstuff (primarily foodstuffs with a genetically modified basis and functional foods or nutraceuticals). We also feel that if assessments are made by a body with the Council's independent scientific status, this will support the validity of the Netherlands' opinion in the eyes of the European Committee and other member states.

My wish is to make the procedure and the assessment as open and transparent as possible, so as to enhance consumer trust in the safety of novel foods. I would like the Health Council to support this

objective by, for example, allowing perusal of the application dossier (insofar as consistent with the need to protect the confidentiality of commercially sensitive information) and publishing the criteria upon which safety assessments are made.

The Minister of Health, Welfare and Sport,
(signed) dr E Borst-Eilers

Annex **B**

The Committee

- Prof. dr LM Schoonhoven, *chairman*
  emeritus professor of entomology; Wageningen University and Research centre
- Prof. dr CAFM Bruijnzeel-Koomen
  professor of dermatology/allergology; Academic Hospital Utrecht
- Ir EJ Kok
  toxicologist; National Institute for Quality Control of Agricultural Products, Wageningen
- Dr CF van Kreijl
  molecular biologist; National Institute of Public Health and the Environment, Bilthoven
- Prof. dr P van der Laan
  professor of statistics; Technical University Eindhoven
- Dr B Loos, *advisor*
  Committee on Genetic Modification, The Hague
- Prof. dr FM Nagengast
  gastro enterologist; Academic Hospital Nijmegen
- Dr ir JMA van Raaij
  food physiologist; Wageningen University and Research centre
- Prof. dr ir G Schaafsma
  professor of nutrition; TNO Nutrition and Food Research, Zeist
- Prof. dr EG Schouten
  professor of epidemiology; Wageningen University and Research centre

- Dr GJA Speijers
  toxicologist; National Institute of Public Health and the Environment, Bilthoven
- Prof. Dr WJ Stiekema
  professor of bioinformatics; Wageningen University and Research centre
- Ir R Top, *advisor*
  Ministry of Health, Welfare and Sport; The Hague
- Prof. dr WM de Vos
  professor of microbiology; Wageningen University and Research centre
- Dr RA Woutersen
  toxicologist; TNO Nutrition and Food Research, Zeist
- Dr CMA van Rossum, *scientific staff member*
  Health Council of the Netherlands, The Hague

Administrative assistance: AD Lugtenburg; Health Council of the Netherlands, The Hague
Lay-out: J van Kan; Health Council of the Netherlands, The Hague

Annex C

EU-procedure

When manufacturers bring novel foodstuffs onto the market, consumer safety has to be ensured. In 1997, a European Regulation (EC97) came into force, laying down the procedure for approving the market introduction of novel foodstuffs. The procedure recognizes various actors. The applicant must decide whether a product is a novel foodstuff, i.e. a substance that has not previously been available for human consumption to any substantial extent within the European Union and is not substantially equivalent to any existing product. (If a foodstuff is substantially equivalent to any existing product, it is sufficient to inform the authorities of its market introduction). Food additives, aromas and extracts are excluded from the provisions of the directive, since they fall within the scope of an established assessment regime. Before marketing a novel foodstuff, the applicant must compile a safety dossier that complies with the Recommendations of the European Commission (EC97a). These Recommendations are based on reports by a number of bodies that have studied the issue of novel foodstuffs, in particular the OECD (OECD93, OECD96) and the WHO/FAO (FAO96, WHO91). The Health Council of the Netherlands has also considered the question earlier (HCN92). Since publication of the EU recommendations, international efforts have been made to clarify and adapt the latest scientific knowledge in the field (FAO01, OECD98, OECD00, SCF99, SSC99, WHO00).

Having compiled a dossier in line with the guidelines, the manufacturer has to submit it to the competent authority in the country where the product is to be marketed first. This dossier is assessed by the national safety assessment authority. In the

Netherlands, this is the Minister of Health, Welfare and Sport, who is advised by the Health Council. The President of the Health Council has created a Committee on the Safety Assessment of Novel Foods (VNV Committee) to advise the minister on behalf of the Council.

On the basis of the scientific state of the art, the committee has to decide whether the information provided by the manufacturer is accurate and complete and whether the manufacturer's conclusions are sound. The committee then draws up a report on its findings for the minister; this report must also comply with the European Recommendation (EC97a, part III). After considering the report, the minister formulates the Netherlands' opinion regarding the foodstuff in question, which is discussed at European level in the Standing Committee on Foodstuffs. All other European member states are invited to express a 'second opinion' regarding the dossier and the first opinion. The Standing Committee then arrives at a final judgement. If a dossier is particularly contentious, the European Commission calls upon the Scientific Committee on Food for advice. If consensus still cannot be reached, the issue is referred to the European Council of Ministers.

Annex **D**

Executive summary of the dossier

Application under Regulation (EC) No. 258/97 concerning Novel Foods and Novel Food Ingredients to the Minsterie van Volksgezondheid, Welzijn en Sport of the Netherlands

for

The Evaluation of the Safety and Use of Foods and Food Ingredients derived from Roundup Ready® Maize Line NK603

Summary

This application is for authorisation under Regulation (EC) Number 258/97 on Novel Foods and Food Ingredients, for the placing on the market of grain and derived ingredients of Roundup Ready®[1] maize line NK603. As part of this application, an opinion of substantial equivalence is sought for those ingredients which may be considered for notification under the provisions of Article 5 of the said Regulation.

Roundup Ready maize line NK603 has been developed by Monsanto Company. This product has tolerance to Roundup®[1] herbicide (containing glyphosate), which is an approved herbicide of Monsanto Company, and allows the agronomic use of Roundup in maize for broad spectrum, post-emergence weed control. Maize line NK603 was produced by the introduction of a glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). The genetic modification, produced by particle acceleration technology with an agarose gel-isolated *Mlu*I restriction fragment of plasmid PV-ZMGT32 resulted in the stable integration of this fragment into the maize genome. By design, the *Mlu*I fragment of vector PV-ZMGT32 contained two CP4 EPSPS gene cassettes, one utilising a rice actin (ract) promoter while the second utilised an enhanced cauliflower mosaic virus promoter (e35S). The Roundup Ready maize line does not contain any elements of the plasmid backbone sequence.

Maize, *Zea mays* L., has a history of safe use as a source of food in the EU. Therefore, information is provided according to the requirements for this class of Novel Foods and Food Ingredients (Class 3.1), as described in the Commission Recommendation (97/618/EC) of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No. 258/97.

[1] Roundup Ready® and Roundup® are registered trademarks of Monsanto Company.

The scientific approach to the safety assessment of Roundup Ready maize line NK603 was based on the characteristics of the CP4 EPSPS protein and the principle of substantial equivalence defined by the Organisation for Economic Cooperation and Development (OECD) and presented according to the guidelines 'Opinion of the Scientific Committee for Food on the Safety Assessment of Novel Foods'. The following points were considered in the safety assessment:

1. Maize (*Zea mays* L.), the Novel Food source, has a long history of production and safe use world-wide and within the EU.

2. Maize line NK603 was genetically modified to be tolerant to glyphosate, the active ingredient in Roundup herbicide. The introduced trait is of agronomic value and will not affect the extent of intake or extent of use of food ingredients derived from maize.

3. Agronomic performance, plant morphology, and other phenotypic observations indicate that there are no unintended effects resulting from the genetic modification. The genetic modification is stably inherited and expressed in subsequent generations.

4. The results of two years of compositional analysis of maize line NK603 demonstrate that the levels of the major nutrients are comparable to those of the non-transgenic control as well as to maize varieties in commerce. The data demonstrate that, with the exception of the introduced DNA insert and resulting CP4 EPSPS protein produced, maize line NK603 is substantially equivalent to non-transgenic maize, and thus the food ingredients derived from Roundup Ready maize will also be nutritionally equivalent to food ingredients derived from non-transgenic maize. A chicken broiler performance study further confirms the nutritional equivalence of maize line NK603 to a parental control and commercial maize.

5. The introduced protein, CP4 EPSPS, is homologous to EPSPSs derived from food sources with a long history of safe consumption.

6. The CP4 EPSPS protein itself, as produced in Roundup Ready maize line NK603, has a history of safe human and animal consumption based upon the extensive production and use of Roundup Ready soyabean, canola, and cotton expressing the same CP4 EPSPS protein.

7. Additional studies confirm the safety of the CP4 EPSPS protein. The CP4 EPSPS protein is rapidly digested in simulated mammalian gastric and intestinal systems, exhibits no toxicity in acute gavage studies in mice, does not possess the characteristics of known protein allergens, has no homology to known toxins and allergens, and is of very low exposure in the human diet.

Based on information presented in this document, which includes information on safety and extensive compositional analysis, food and food ingredients derived from Roundup Ready maize line NK603 and its progeny are nutritionally equivalent to food and food ingredients derived from maize currently grown. Following the principles for the application of substantial equivalence, no safety or nutritional concerns of any significance are anticipated.

Food and food ingredients derived from Roundup Ready maize are as safe as food and food ingredients produced from non-transgenic maize. Therefore, Roundup Ready maize line NK603 and any progeny derived from crosses between this line and other maize lines may be used in the same manner as conventional maize.

A detailed description of the Roundup Ready maize line NK603 follows in this submission.

Application under Regulation (EC) No. 258/97 concerning Novel Foods and Novel Food Ingredients to the *Ministerie van Volksgezondheid, Welzijn en Sport* of the Netherlands

for

The Evaluation of the Safety and Use of Foods and Food Ingredients derived from NK603 Roundup Ready® Maize

Summary

This application is for authorisation under Regulation (EC) Number 258/97 on Novel Foods and Food Ingredients, for the placing on the market of grain and derived ingredients of NK603 Roundup Ready®[1] maize. As part of this application, an opinion of substantial equivalence is sought for those ingredients, which may be considered for notification under the provisions of Article 5 of the said Regulation.

NK603 Roundup Ready maize has been developed by Monsanto Company. This product has tolerance to Roundup®[1] herbicide (containing glyphosate), which is an approved herbicide of Monsanto Company, and allows the agronomic use of Roundup in maize for broad spectrum, post-emergence weed control. NK603 maize was produced by the introduction of a glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). The genetic modification, produced by particle acceleration technology with an agarose gel-isolated *Mlu*I restriction fragment of plasmid PV-ZMGT32, resulted in the stable integration of this fragment into the maize genome. By design, the *Mlu*I fragment of vector PV-ZMGT32 contained two *cp4 epsps* gene cassettes, one utilising a rice actin (*ract*) promoter while the second utilised an enhanced cauliflower mosaic virus promoter (*e35S*). NK603 Roundup Ready maize does not contain any elements of the plasmid backbone sequence.

Maize, *Zea mays* L., has a history of safe use as a source of food in the E.U. Therefore, information is provided according to the requirements for this class of Novel Foods and Food Ingredients (Class 3.1), as described in the Commission Recommendation (97/618/EC) of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No. 258/97.

The scientific approach to the safety assessment of NK603 Roundup Ready maize was based on the characteristics of the CP4 EPSPS proteins and the principle of substantial equivalence defined by the Organisation for Economic Cooperation and Development (OECD) and presented according to

[1] Roundup Ready and Roundup are registered trademarks of Monsanto Technology LLC.

the guidelines 'Opinion of the Scientific Committee for Food on the Safety Assessment of Novel Foods'. The following points were considered in the safety assessment:

1. Maize (*Zea mays* L.), the Novel Food source, has a long history of production and safe use worldwide and within the E.U.
2. NK603 maize was genetically modified to be tolerant to glyphosate, the active ingredient in Roundup herbicide. The introduced trait is of agronomic value and will not affect the extent of intake or extent of use of food ingredients derived from maize.
3. Agronomic performance, plant morphology, and other phenotypic observations indicate that there are no unintended effects resulting from the genetic modification. The genetic modification is stably inherited and expressed in subsequent generations.
4. The results of two years of compositional analysis of NK603 maize demonstrate that the levels of the major nutrients are comparable to those of the nontransgenic control as well as to maize varieties in commerce. The data demonstrate that, with the exception of the introduced DNA insert and resulting CP4 EPSPS proteins produced, NK603 maize is substantially equivalent to nontransgenic maize, and thus the food ingredients derived from NK603 Roundup Ready maize will also be nutritionally equivalent to food ingredients derived from nontransgenic maize. A chicken broiler performance study further confirms the nutritional equivalence of NK603 maize to a parental control and commercial maize.
5. The introduced CP4 EPSPS proteins are homologous to EPSPSs derived from food sources with a long history of safe consumption.
6. The CP4 EPSPS protein itself, as produced in NK603 Roundup Ready maize, has a history of safe human and animal consumption based upon the extensive production and use of Roundup Ready soybean, canola, and cotton expressing the same CP4 EPSPS protein.
7. Additional studies confirm the safety of the CP4 EPSPS proteins. The CP4 EPSPS proteins are rapidly digested in simulated mammalian gastric and intestinal systems, CP4 EPSPS exhibits no toxicity in acute gavage studies in mice, these proteins do not possess the characteristics of known protein allergens, have no homology to known toxins and allergens, and are of very low exposure in the human diet.

Based on information presented in this document, which includes information on safety and extensive compositional analysis, food and food ingredients derived from NK603 Roundup Ready maize and its progeny are nutritionally equivalent to food and food ingredients derived from maize currently grown. Following the principles for the application of substantial equivalence, no safety or nutritional concerns of any significance are anticipated.

Food and food ingredients derived from NK603 Roundup Ready maize are as safe as food and food ingredients produced from nontransgenic maize. Therefore, NK603 Roundup Ready maize and any progeny derived from

crosses between this variety and other maize varieties may be used in the same manner as conventional maize.

A detailed description of NK603 Roundup Ready maize follows in this submission.