# Assessment Report of the Robert Koch Institut in Accordance with Directive 2001/18/EC

8 April 2003

# Insect-Resistant Maize MON 863 and MON 863 X MON 810

Application by Monsanto Company, USA,
Represented by Monsanto Europe S.A., Brussels, Belgium,
for the Placing on the Market of Genetically Modified Maize
in Accordance with Directive 2001/18/EC

# Contents

# 1 Introduction

On 13 August 2002, the Monsanto Company, USA, represented by Monsanto Europe S.A., 270–272 Avenue de Tervuren, B-1150 Brussels, Belgium, submitted an application to the Robert Koch Institut (RKI) for a registration of genetically modified maize (*Zea mays*) MON 863 and MON 863 X MON 810 in accordance with Directive 2001/18/EC, for the maximum possible registration term of ten years.

The initial examination of the application document by the RKI led to the conclusion that it was not complete, with the result that additional documents had to be submitted. Following submission of these documents by the applicant in December 2002 and in January and February 2003, the application was declared by the RKI to be complete in accordance with the provisions of Directive 2001/18/EC on 14 February 2003

A summary of the application document (SNIF) was published on 17 February 2003 on the Internet site of the Joint Research Center of the EU.

The selection, presentation and quality of the data available in the full version of the application are in accordance with Directive 2001/18/EC. In the view of the RKI, the application is adequate and appropriate, allowing an assessment of the products intended to be introduced to be performed. The investigations were performed in accordance with internationally accepted methods to the extent that such methods are available. A validity and plausibility check has been carried out. The interpretations and assessments of the applicant with regard to the information and data relevant to the safety assessment are adequately documented and presented in a clearly understandable and conclusive manner, and they are shared by the RKI.

Several documents were declared by the applicant to be confidential in accordance with Art. 25 of Directive 2001/18/EC. The applicant stated that the dissemination of this information could harm his competitive position. In the view of the RKI, the information provided by the applicant fulfils the requirements of Directive 2001/18/EC. There is no apparent ground for refusal.

The applicant declared his willingness to inform traders and other parties involved in the use of the products that the products are subject to the labelling and traceability requirements of Directive 2001/18/EC, and that the products must comply with possible additional requirements for labelling and traceability arising from European legislation ('any Community legislation adopted to regulate the traceability and labelling of transgenic organisms').

As a proposal for a label in accordance with Art. 13, Sect. 2 f) of Directive 2001/18/EC, the applicant intends to put the text 'This product contains genetically modified organisms' on the label and/or accompanying papers.

There is also a provision for giving information on the unique identifiers. A proposal has been made that is in agreement with the rules established by the OECD: 'MON-ØØ863-5' for MON 863 and 'MON-ØØ810-6' for MON 810. For the MON 863 X MON 810 hybrid, a combination of both unique identifiers is proposed.

Additional relevant product information, such as trade names and information about the official register of the EU, are also to be conveyed to the traders.

For detecting MON 863, the applicant has supplied information about a PCR-based 'event'-specific assay method. The applicant also stated his willingness to assist in the development of a validated identification method by the Joint Research Center (JRC) of the EU, by providing MON 863 reference material, methods and sequence information for the associated PCR primers.

For MON 810, an event-specific assay method has already been published (2002/66/EC – ABL. EC No. 26, 30 January 2002, p. 8). Further studies on the validation of methods are currently being carried out. The applicant has already provided the JRC with sufficient quantities of MON 810 reference material for this purpose.

In a cover letter on the occasion of the provision of supplementary documents in support of the application (December 2002), the applicant stated that suitable reference material will be provided to the RKI in June 2003.

## 2 Subject of the Application

The subject of the application is the placing on the market of maize kernels from the progeny of the genetically modified maize line MON 863, which is resistant to certain coleoptera, in particular the larvae of maize root borers (*Diabrotica* sp.), as well the placing on the market of maize kernels of the hybrid maize MON 863 X MON 810 produced by conventional crossing of the two genetically modified maize lines MON 863 and MON 810, which combines resistance to coleoptera with resistance to specific lepidoptera species such as the European corn borer (*Ostrina nubilalis*).

Maize kernels are to be imported into the European Union for use as animal feed and for further processing. The present application does not include cultivation within the EU or using products made from genetically modified maize kernels for human nutrition.

An unrestricted registration of the progeny of the genetically modified maize line MON 810 and products made from these progeny into the European market (Notification C/F/95/12-02), based on Directive 90/220/EEC, was granted by means of European Commission Resolution 98/294/EC of 22 April 1998 and publication of the decision of the responsible French authority on 05/08/1998.

## 3 Assessment of the Submitted Data and Documents

For the assessment of the present application, based on the results of constituent analyses, animal feeding experiments, characterisation of agronomic and morphological properties and the investigation of additional phenotypic parameters, it was concluded for the genetically modified organism and products obtained from it, that with the exception of the expression of the gene newly transferred into the recipient plants and the associated content of cry proteins and NPT II, there would be substantial equivalence with corresponding conventional products. Conventional maize, for which there are many years of experience with regard to cultivation and use as a raw material for foods and animal feeds, can be employed as a comparative reference.

Based on the concept of substantial equivalence as a starting point for the safety assessment, in the view of the RKI it is both possible and meaningful to concentrate the testing and assessment of the products on the newly transferred features and properties.

### 3.1 Information regarding the recipient organism and the use of maize

With regard to the recipient organism, maize, there are many years of experience with its cultivation and processing as a raw material for animal feeds and foods. The genetic modifications relate to agronomic properties and do not have the objective of modifying its nutritional properties or compositions or modifying the use of maize as a raw material.

It was not necessary to reassess the processing paths of maize, since neither changes to the processing nor new products are to be expected, and there are many years of accumulated experience in handling maize.

## 3.2 Description of the genetically modified organisms

### 3.2.1 MON 863

Using the particle acceleration method, the recipient maize line AT, cell line AT824, was transformed using the 4691-bp *Mlu*I fragment PV-ZMIR13L of the pUC plasmid derivative PV-ZMIR13. The desired fragment, was isolated using agarose gel electrophoresis and purified. On this fragment, in addition to an *npt*II gene from *E. coli Tn5* under control of the 35S promoter of the cauliflower mosaic virus (CaMV) and the terminator region of the *nos* gene from *Agrobacterium tumefaciens*, there is a gene that provides resistance against coleoptera (*MON 863 cry3Bb1*). This gene is a synthetic variant of *cry3Bb* from *Bacillus thuringiensis* ssp. *kumamotoensis*, with a DNA sequence that deviates from that of the wild type. With respect to the amino acid sequence of the original Cry3Bb1 protein, the MON 863 cry3Bb1 version coded from the synthetic gene variant is distinguished by an additional alanine at position 2 and six additional amino acid changes (D166G, H232R, S312L, N314T, E318K and Q349R), which results in increased toxicity to the target organisms.

The *MON 863 cry3Bb1* gene is under the control of a fourfold copy of a 21 bp long portion of the CaMV 35S promoter that is designated AS1 (activating sequence 1), in combination with an additional portion of the 35S promoter (4AS1 promoter). After this comes the 5' non-translated leader region *wt CAB* of the chlorophyll a/b binding protein from *Triticum aestivum* as a translation enhancer, followed by the first intron of the *actin 1* gene from *Oryza sativa* as a transcription enhancer. The *MON 863 cry3Bb1* gene is terminated by the 3' non-translated region of the heat shock protein 17.3 from *Triticum aestivum* (see Figure 1).

In the 3' region of the *npt*II gene, there is a fragment on PV-ZMIR13L that codes for 51 aminoterminal amino acids of the *ble* gene (bleomycin binding protein, *Tn5*) out of a total of 121 amino acids. The open reading frame of the fragment begins 20 nt after the stop codon of *npt*II. Together with a polylinker and portions of the *nos* terminator, this fragment forms an open reading frame that could code for a total of 89 amino acids. This would yield a protein with a molecular weight of 10.25 kDa, which is designated BLE 10.25 in the application. The open reading frame for the *ble* fragment is not located in the same reading frame as the *npt*II gene.

Based on theoretical considerations, a translation of this open reading frame either by itself or as a fusion protein with NPT II appears improbable. Natural ribosome binding sites, such as IRES (internal ribosome entry sites), were not identified, and read-through of ribosomes from the *npt*II transcript into the open reading frame of BLE 10.25 is not to be expected, due to the different reading frames.

Western Blot analyses with antibodies against the BLE portion of the hypothetical protein produced no evidence of the expression of the ORF (with a detection limit of 1.7 µg/g fresh mass).

Should BLE 10.25 actually be formed in small quantities, despite theoretical considerations and ELISA results, a functionality, which means the ability to bind and thus deactivate bleomycin (glycopeptide antibiotic from *Streptomyces verticillus*), appears improbable. The natural protein BLE becomes effective as a homodimer and does not have any enzymatic activity. The protein only binds bleomycin, thereby disabling its DNA-splitting effect. Since the

truncated BLE 10.25 lacks essential regions for dimerisation, it is unlikely that it would have the capacity to bind bleomycin.

Figure 1: Schematic representation of the insert transferred to the recipient plants in the transformation of MON 863 (source: application)

Using segregation analyses and investigations of the genomic DNA of MON 863 by means of Southern Blot analyses, PCR and DNA sequencing, it was possible to show that one (1) copy of the transferred fragment was integrated at one (1) location in the maize genome and that it was inherited stably in the progeny. Supplementary analyses using various restriction enzymes and probes confirmed that the gene is present on the transferred fragment in the intended form. No other sequences, in particular no sequences of the pUC plasmid backbone, were found.

PCR amplicons from genomic DNA of the MON 863 maize line representing overlaps of the two ends of the insert with the flanking sequences were sequenced. At the 5' end, a 508-bp region was amplified with a flanking region of 242 bp length not belonging to the insert. At the 3' end, 584 bp were amplified with a flanking region of 224 bp length. It was shown that the sequence of the insert in MON 863 corresponds to the region from position 7 to 4681 of the introduced PV-ZMIR143L fragment, so that all functional regions were transferred in the process of transformation. For this reason, it was concluded that two new proteins, MON 863 Cry3Bb1 and NPTII, are expressed in their full length in the MON 863 maize line as a result of the genetic modification. (see 'Expression analysis').

A comparison of the sequences of the flanking regions with public sequence databases yields a 99 percent homology between the 5' region and exon 4 of the mitochondrially coded *Zea mays* NADH dehydrogenase subunit (*nad4*). It is possible that integration of mitochondrial sequences may have occurred during transformation.

For the sequences in the 3' flanking region, no striking homologies with DNA sequences in public data base were found.

Bioinformatic analysis of the amino acid sequences performed by comparing all possible open reading frames of the transition regions from the insert into the flanking regions with suitable protein databases did not yield any indications of structural or immunological similarities to known allergens, toxins or pharmacologically active proteins.

### 3.2.2 MON 810

Using the particle acceleration method, the recipient maize Hi-II, which is derived from the inbred lines A188 and B73, was transformed using the plasmid pV-ZMBK07, a pUC19 derivative. Besides the origin of replication (*ori*) and the *lac* sequences, the plasmid contains the *npt*II gene of the *Tn5* transponson from *E. coli,* under the control of its own promoter.

In addition to this, the plasmid pV-ZMBK07 contains the *cry*IA(b) gene for a δ-endotoxin from the *Bacillus thuringiensis* ssp. *kurstaki* HD1 strain. The *cry*IA(b) gene consists of 3468 base pairs and codes for a protein made up of 1156 amino acids. In order to optimise expression in maize, the gene is adapted to the normal codon usage in plants, and the intron sequence of the maize *hsp*70 gene (heat shock protein) is inserted ahead of the δ-endotoxin coding region. Expression of the δ-endotoxin gene in the genetically modified plants is controlled by a 35S promoter from CaMV with a double enhancer region (E35S). The non-translated DNA sequence (*nos* 3') of the nopaline-synthase gene from *Agrobacterium tumefaciens* was used as a transcription terminator.

In the MON 810 transformant, of the described regions of the pV-ZMBK07 plasmid only one (1) copy of the *cry*IA(b) gene truncated at the 3' end together with the E35S promoter and the *hsp*70 intron was integrated at one (1) locus of the maize genome (see Figure 2). Amino acids 1 through 816 are coded from the truncated gene. The open reading frame continues in the adjacent region of the genome and codes for two additional amino acids (phenylalanine and arginine). It is followed by a stop codon, resulting in the formation of a protein made up of 818 amino acids in total.

The stable chromosomal integration of the transferred *cry1*Ab gene was demonstrated by segregation analyses and investigations of the genomic DNA of MON 810 using Southern Blot analyses.

Southern Blot analyses were also used to show that the *npt*II and *ori* sequences present on the vector used for the transformation are not transferred into the recipient plant. Moreover, NPT II could not be detected using Western Blot analyses.

Since the truncated *cry*IA(b) gene from *Bacillus thuringiensis* ssp. *kurstaki* that is transferred into the genome of the MON 810 maize line is transcribed based on a 35S promoter, constitutive expression in the plant is expected (see 'Expression analysis').

The DNA sequences of the overlaps of the two ends of the insert with the flanking regions were submitted by the applicant. Information was provided for a flanking region with a length of 244 bp at the 5' end and a 195-bp region at the 3' end.

Figure 2: Schematic representation of the insert transferred into the recipient plant in the transformation of MON 810 (source: application)

For the 3' region, a sequence comparison of the flanking region with public sequence databases yielded homologies to the mitochondrially coded ribosomal protein 13 (*rps*13) and the closely linked gene in the mtDNA for the Fo ATPase subunit 9, which suggests that co-integration of mitochondrial DNA may have occurred during transformation.

For the sequences in the 5' flanking region, there are indications in the databases of homologies with various genomic maize DNA sequences, in particular zein genes.

Bioinformatic analysis of the amino acid sequences performed by comparing all possible open reading frames of the 3' transition region from the insert into the flanking region using suitable protein databases did not yield any indications of structural or immunological similarities to known allergens, toxins or pharmacologically active proteins. Only the open reading frame of Cry 1Ab has been identified and the comparison with toxin databases yielded homologies only to other Cry toxins.

### 3.2.3 Expression analysis of Cry proteins and NPT II

Plant samples from several locations in the USA (1999) and Argentina (1999/2000) were investigated for concentrations of MON 863 Cry3Bb1, Cry1A(b) and NPTII using ELISA. The results are presented in summary form in Tables 1 through 4. The highest average expression values are printed in bold. Investigations of the expression MON 863 Cry3Bb1 and NPTII in leaves, entire plants and roots during the course of a vegetation period showed a decline in protein concentrations relative to the fresh mass in all investigated tissues (data not shown).

Table 1: **MON 863 Cry3Bb1** and **NPT II** protein content in µg/g fresh mass in plant tissues of MON 863 from field trials conducted in 1999 in the USA

| Tissue type | Days post-planting | Cry3Bb1 (range) | NPT II (range) |
|---|---|---|---|
| Young leaf | 21 | **81** (65–93) | **0.98** (0.74–1.4) |
| Forage | 90 | 39 (24–45) | 0.19 (0.17–0.23) |
| Mature root | 90 | 41 (25–56) | not done |
| Grain | 125 | 70 (49–86) | <0.076 (LOD) |
| Silk | 58 | 10 (1 sample) | not done |
| Pollen | 60 | 62 (30–93) | not done |

Table 2: **MON 863 Cry3Bb1** protein content in µg/g fresh mass in plant tissues of MON 863 X MON 810 from field trials conducted in 1999/2000 in Argentina

| Tissue type | Days post-planting | MON 863 X MON 810 | MON 863 |
|---|---|---|---|
| Young leaf | 18 | 46.7 (35.5–53.2) | 30.0 (21.3–47.2) |
| Pollen | 60 | **79.6** (65.1–96.5) | **60.4** (29.7–90.7) |
| Forage | 90 | 23.6 (6.7–39.7) | 12.8 (<0.22–28.8) |
| Grain | 117 | 61.1 (38.5–83.1) | 43.7 (<0.096–84.1) |

Table 3: **MON 863 Cry1A(b)** protein content in µg/g fresh mass in plant tissues of MON 863 X MON 810 and MON 810 from field trials conducted in 1999/2000 in Argentina

| Tissue type | Days post-planting | MON 863 X MON 810 | MON 810 |
|---|---|---|---|
| Young leaf | 18 | **17.9** (14.1–27.5) | **13.0** (1.5) |
| Pollen | 60 | <0.08 (<0.08–0.18) | <0.08 (<0.08) |
| Forage | 90 | 7.9 (3.9–11.9) | 5.6 (3.0–8.2) |
| Grain | 117 | 0.84 (0.63–1.2) | 0.46 (0.24–0.77) |

Table 4: **NPTII** protein content in µg/g fresh mass in plant tissues of MON 863 X MON 810 and MON 810 from field trials conducted in 1999 in the USA

| Tissue type | n | MON 863 X MON 810 | MON 863 |
|---|---|---|---|
| Young leaf | 18 | **1.60** (0.53–2.32) | **1.05** (0.58–1.56) |
| Forage | 90 | 0.19 (0.13–0.27) | 0.17 (<0.075–0.33) |
| Grain | 117 | <0.076 (LOD) | <0.076 (LOD) |

### 3.3 Experience from previous field trial investigations

In the European Union, there have not been any releases of MON 863 or of the hybrid MON 863 X MON 810.

The genetically modified maize line MON 863 has been released in the USA, Canada, Chile, Argentina, and Japan. The hybrid MON 863 X MON 810 has been studied in release trials in the USA and Argentina.

These trials primarily served to generate data for approval procedures and verification of the efficacy of the transferred insect resistance, as well as other agronomic properties. For MON 863, there were no indications of metabolically determined phenotypic differences with regard to various agronomic parameters (plant development, time of flowering, morphology, yield parameters and persistence) compared to controls. There are thus no indications of any modification of MON 863 with regard to survival, propagation and dissemination capacity.

For MON 810, it is stated in the application that based on field trials, the genetically modified maize has the same properties as maize lines that have not been genetically modified, with the exception of resistance to several species of *Lepidoptera*.

In the present application it is concluded, based on the individual investigations of MON 863 and MON 810, that in the case of hybrids of the two, it can be expected that MON 863 X MON 810 also does not change with regard to its survival, propagation or dissemination capacity.

Based on analyses of selected components of MON 863 and the MON 863 X MON 810 hybrid, there are no indications of unintentional effects on the plant metabolism as a result of the genetic modification, either in the sense of a 'position effect' or as the result of pleiotropic effects.

### 3.4 Registration granted outside the E.U.

The MON 863 genetically modified maize line has been authorised in the USA and Japan. The MON 810 genetically modified maize line has been authorised in the EU, Argentina, Australia, Canada, Japan, Korea, the Philippines, South Africa, Switzerland and the USA (source: AGBIOS database).

There is no information available regarding the international registration status of hybrids produced by conventional crossing. However, a separate registration for introducing hybrids of approved GMOs is not required in all countries.

### 3.5 Assessment of use in animal feeds

A risk assessment with respect to the use of products of the genetically modified maize plants for the production of animal feed requires assessing modifications to the composition of the genetically modified maize plants brought about by the transferred DNA segments, including the newly formed proteins, possible modifications of the compositions resulting from context modifications and possible horizontal gene transfer to microorganisms in the gastro-intestinal tract.

### 3.5.1 Assessment of the newly formed proteins

The assessment of the toxicological and allergenic properties of the novel foodstuff is substantially based on investigations of the newly formed proteins. Such an assessment is possible because other investigations of the composition of MON 863 and MON 863 X MON 810 have not yielded any biologically relevant differences from conventional maize with regard to compositions or to phenotypic or physiological parameters.

#### 3.5.1.1 MON 863 (NPTII, MON 863 Cry 3Bb1)

In order to confirm the identity of the Cry3Bb1 expressed in MON 863 by comparison with the amino acid sequence derived from the DNA sequence, protein extracts purified by immunoaffinity chromatography were investigated using N-terminal sequencing and MALDI TOF mass spectroscopy performed following trypsin digestion. This method allows theoretically derived fragments to be compared with actually found fragments on the basis of molecular mass determinations. The fragments identified using MALDI TOF MS (molecular masses) correspond to the postulated fragments of the MON 863 Cry3Bb1 protein. The results provide strong evidence of agreement between the Cry3Bb1 protein expressed in MON 863 and the predicted protein.

In the N-terminal amino acid sequencing of the 74 kDa protein from MON 863, in contrast to the corresponding protein resulting from microbial production, there was no usable result (blocking). This is interpreted by the applicant to mean that a post-translational modification in the aminoterminal region may have occurred (N-terminal acetylation).

In a letter accompanying an additional delivery of information (December 2002), the applicant states that he is not aware of any data or scientific publications reporting that non-toxic or non-allergenic proteins had developed toxic or allergenic potential as a result of N-terminal acetylation.

The RKI also concludes that it is unlikely that this protein modification will have any significant effect on physico-chemical properties. There is no discussion of posttranslational N-terminal acetylation in connection with allergenic characteristics (although there is discussion of glycosylation).

The functional and biochemical equivalence of Cry3Bb1 protein produced bacterially in *E. coli* and vegetatively in MON 863 was demonstrated by means of MALDI TOF mass spectroscopy, N-terminal sequence analysis (not blocked by N-acetylation, since they begin with partially decomposed N-termini), immunoblot, insect bioassay, SDS-PAGE, analysis of glycosylation and determination of amino acid composition.

For the NPTII protein, immunoblot tests were used to demonstrate the equivalence of the proteins from MON 863 and *E. coli* with regard to molecular weight (approx. 29 kDa) and immunological reactivity with respect to monoclonal antibodies.

The results of studies performed using bacterially produced proteins can thus be applied to the safety assessment of MON 863.

NPTII

The neomycin phosphotransferase is a type II aminoglycoside-3'-phosphotransferase (APH(3')II = NPT II) that catalyses the ATP-dependent phosphorylation of the 3'-hydroxyl group of the aminohexose ring of certain aminoglycoside antibiotics, and thereby inactivates them. The enzyme is distinguished by a high substrate specificity (Nap *et al.*, 1992). Catalytic activity of the enzyme in the gastrointestinal tract of mammals depends on the availability of substrates (antibiotics and ATP), as well as suitable reaction conditions.

With regard to NPT II, the *Stellungnahme der ZKBS zur biologischen Sicherheit von Antibiotika-Resistenzgenen im Genom gentechnisch veränderter Pflanzen* ['Report of the ZKBS on the Biological Safety of Antibiotic Resistance Genes in the Genome of Genetically modified Plants'] of 1999 states, 'The substrates of the APH(3')II enzyme include the antibiotics kanamycin, neomycin, geneticin, butirosin, gentamicin A & B and paramomycin. Amikacin and gentamicin (primarily C1, C1$\alpha$ and C2), which are therapeutically significant in human medicine, as well as sundry aminoglycosides and aminocyclitoles, do not belong to the substrate spectrum of the APH(3')II enzyme (Trieu-Cuot *et al.*, 1987; Davies, 1991; Simon and Stille, 1989).'

Although they are in part approved for use in Europe, the potential substrates of NPT II, such as neomycin, kanamycin, gentamicin A & B and paramomycin, are presently only of minor significance in human and veterinary medicine, due to their toxicity and/or unfavourable resistance situations, or their use (in the case of paramomycin) is limited to very specific types of infection (Kroker *et al.*, 2002).

Consequently, due to the simple lack of substrates, it is not expected that there will be large-scale formation of new, potentially harmful reaction products in the digestive tract due to the enzymatic activity of NPT II protein ingested with the fodder.

In a **comparison of the amino acid sequence of the NPT II protein** with **known toxins and pharmacologically active substances** using database searches with a total of 4677 protein sequences, no biologically relevant similarities to mammalian toxic or pharmacologically active proteins were discovered. Based on this investigation, there are thus no indications of a toxic potential from NPT II.

The **acute toxicity** of NPT II was tested using a protein produced in *E. coli* (Fuchs *et al.*, 1993). The protein was orally administered to albino mice in two successive doses (with a time difference of four hours) at three different dosage levels (100, 1000 and 5000 mg/kg BW), using 10 male and 10 female animals for each dose. Clinical observations were performed, and the evolutions of body weight (BW) and feed consumption were determined. At the end of the test (days 8 and 9), the animals were killed and subjected to necroscopy.

No substance-specific negative effects were found with the oral administration of NPT II protein for dosage levels up to 5000 mg/kg BW. Consequently, the LD50 is > 5000 mg/kg BW and the NOEL is 5000 mg/kg BW.

With an estimated proportion of 10 g of maize kernels per kilogram db in the forage of dairy cows, this yields a daily consumption of approximately 0.001 mg NPT II per kilogram body weight, given a concentration of approximately 0.0001 mg NPT II per gram MON 863 maize kernels. With a NOEL (for mice) of > 5000 mg/kg BW, this yields a safety margin of > 5 000 000 for NPT II in fodder.

The **stability of the proteins with respect to proteolytic enzymes** was investigated *in vitro* in simulated mammalian gastrointestinal fluids (gastric fluid SGF and intestinal fluid SIF). In SGF, complete decomposition of NPT II from *E. coli* occurred within 10 seconds. In SIF, 50 percent of the enzyme was decomposed within 2 to 5 minutes. Enzyme tests showed a nearly complete loss of enzymatic activity after 2 minutes of digestion in SGF or 15 minutes in SIF (Fuchs *et al.*, 1993).

The results of the study showed that rapid decomposition of the proteins under the conditions of the gastrointestinal tracts of mammals can be concluded. However, it should be borne in mind that NPT II in MON 863 maize ingested via feed is enclosed in plant cells, so it can withstand the digestive processes longer than the times determined in these experiments.

For the additional **assessment of the allergenic potential of the NPT II protein**, the amino acid sequence was compared with 567 protein sequences of known allergens and coeliac-inducing proteins (gliadine). No biologically relevant sequence homologies were found. A comparison of all possible combinations of eight sequential amino acids of the Cry3Bb1 protein with the above-mentioned 567 protein sequences did not yield any indications of similarities to epitopes of allergy-inducing proteins.

MON 863 Cry3Bb1

In a **comparison of the amino acid sequence** of the Cry3Bb1 protein expressed in MON 863 with **known toxins and pharmacologically active substances** using database searches with a total of 4677 protein sequences, no biologically relevant similarities to mammalian toxic proteins were found.

Sequence similarities between MON 863 Cry3Bb1 and other insecticidal toxins were found. Nearly all sequentially similar toxins belong to the group of *B.t.* delta-endotoxins. The other homologies to sequences from *Clostridium bifermentans, Caenorhabditis elegans, Vibrio chloerae* and *Bacillus popilliae* that were found, were interpreted as being biologically irrelevant due the lack of indications of mammalian toxic activity.

The **acute toxicity** was tested using MON 863 Cry3Bb1 produced in *E. coli*. The protein was orally administered to albino mice in two successive doses (with a time difference of four hours) at three different dosage levels (400, 1100 and 3200 mg/kg BW), using 10 male and 10 female animals for each dose. Clinical observations were performed, and the change of body weight and feed consumption were determined (days 7 and 14). At the end of the test (day 14), all animals were killed and subjected to necroscopy.

No substance-specific negative effects were found with the oral administration of MON 863 Cry3Bb1 protein for dosage levels up to 3200 mg/kg BW. Consequently, the LD50 is > 3200 mg/kg BW and the NOEL is 3200 mg/kg BW.

With an estimated proportion of 10 g of maize kernels per kilogram of body weight in the forage of dairy cows, this yields a daily consumption of approximately 1 mg MON 863 Cry3Bb1 per kilogram of body weight, given a concentration of approximately 0.1 mg Cry3Bb1 per gram of MON 863 maize kernels. With a NOEL (for mice) of > 3200 mg/kg BW, this yields a safety margin of > 3000 for the toxin in fodder.

The **stability of the proteins with respect to proteolytic enzymes** was investigated *in vitro* in simulated mammalian gastrointestinal fluids (gastric fluid and intestinal fluid). MON 863 Cry3Bb1 from *E. coli* and from maize kernels of the MON 863 transformant was rapidly decomposed under the simulated conditions of human gastric fluid (SGF). Complete decomposition occurred within 15 seconds. With the protein from MON 863, a low molecular-weight fragment of approximately 3 kDa was visible as a weak band in the SDS-PAGE for an incubation time of up to 15 minutes maximum (detection limit 17 ng/lane). The results of the study showed that rapid decomposition of the proteins under the acidic conditions in the stomach can be anticipated. Due to the small size of the more slowly decomposed 3-kDa fragment, no allergenic effect is to be expected, because such small peptides presumably are not able to present the two epitopes, separated by a sufficiently large spacer, that are necessary to trigger an allergic reaction.

Digestion experiments using simulated intestinal fluid (SIF) were not performed with the Cry3Bb1 protein expressed in MON 863. Experiments using a Cry3Bb1 protein from a microbial source that is identical except for two amino acids showed that a stable polypeptide of approximately 59 kDa that retained its biological activity was present up to the end of the test after 24 hours (detection using bioassay with larvae of the potato beetle). Based on the high degree of similarity to the investigated Cry3Bb1 variant, it can be concluded that also MON 863 Cry3Bb1 from the GMO will be degraded in SIF only to a stable, insecticidal fragment (core protein).

For the additional **assessment of the allergenic potential of the MON 863 Cry3Bb1 protein**, the amino acid sequence was compared with 567 protein sequences of known allergens and coeliac-inducing proteins (gliadine). No biologically relevant sequence homologies were found. A comparison of all possible combinations of eight sequential amino acids of the Cry3Bb1 protein with the above-mentioned 567 protein sequences did not yield any indications of similarities to epitopes of allergy-inducing proteins.

Altogether, the available information about the properties of the MON 863 Cry3Bb1 and NPT II proteins, as well as the results of feeding studies with mice, rats and chickens (see below) do not provide any reason to anticipate that harmful effects would arise from feeding animals these proteins as components of the genetically modified MON 863 maize.

### 3.5.1.2 MON 810

From the production and use of conventional insecticides based on B.t toxins, many years of experience are available with regard to their toxicological and allergenic properties, including those of the Cry1A(b) protein. Besides this, for the assessment of the Cry1A(b) protein, the applicant has submitted investigations and studies of stability, toxicity and allergenic characteristics that have already been assessed in the course of the authorization procedure for introducing the MON 810 line (Az.6788-02-13).

There are no indications of toxicity with respect to birds, mammals or humans, and such effects are not to be expected, since these taxa lack the receptors necessary for binding the Cry1A(b) protein to gastrointestinal cells.

A **comparison of the amino acid sequence** of the Cry1A(b) protein expressed in MON 810 with **known toxins** in the PIR, EMBL, Swissprot and Genbank databases did not yield any biologically relevant similarities to known toxins, with the exception of homologies to other known *B.t.* proteins.

The **acute toxicity** was tested using Cry1A(b) produced in *E. coli*. Since it can be concluded that the protoxin will be decomposed into a trypsin-resistant core protein during digestion, this core protein was used for the investigations.

The protein was orally administered to albino mice in two successive doses (with a time difference of three hours) at three different dosage levels (400, 1000 and 4000 mg/kg BW), using 10 male and 10 female animals for each dose. Clinical observations were performed, and the evolutions of body weight and feed consumption were determined. At the end of the test (days 8 and 9), all animals were killed and subjected to necroscopy.

No substance-specific negative effects were found with the oral administration of Cry1A(b) protein (trypsinised nuclear protein) for dosage levels up to 4000 mg/kg BW. Consequently, the LD50 is > 4000 mg/kg BW and the NOEL is 4000 mg/kg BW.

With an estimated proportion of 10 g of maize kernels per kilogram of body weight in the forage of dairy cows, this yields a daily consumption of approximately 0.01 mg Cry1A(b) per kilogram of body weight, given a concentration of approximately 0.001 mg Cry1A(b) per gram of MON 863 x MON 810 maize kernels. With a NOEL (for mice) of > 4000 mg/kg BW, this yields a safety margin of > 400 000 for the toxin in animal fodder.

The results of *in vitro* **investigations of stability with respect to simulated digestive fluids** (SGF and SIF) showed that, the Cry1A(b) protein is sensitive to proteolytic enzymes in gastric fluid (SGF) and is decomposed within a few minutes (> 90 percent decomposition within 2 minutes). However, the protein was resistant to decomposition by intestinal enzymes (SIF) (no significant decomposition of the *trypsin-resistant nuclear protein* after 19.5 hours), which can be attributed to the differences in the composition of the simulated digestive fluids with regard to the proteolytic enzymes.

For the additional **assessment of the allergenic potential** of the MON 810 Cry1A(b) protein, the amino acid sequence was compared with 219 protein sequences of known allergens. No biologically relevant sequence homologies were found. A comparison of all possible combinations of eight sequential amino acids of the Cry1A(b) protein with the above-mentioned 219 protein sequences did not yield any indications of similarities to the epitopes of known allergy-inducing proteins.

Altogether, the available information on the properties of the Cry1A(b) protein, as well as the results of feeding studies and constituent analyses (see following section), do not provide any reason to conclude that harmful effects to the health of animals are to be expected from using genetically modified maize kernels from the MON 810 line to produce animal feed. The traditional agricultural use of *Bacillus thuringiensis* preparations, which consist of a mixture of spores and parasporal crystals with δ endotoxins, including Cry1A(b), has not given rise to any indications of hazards to health.

### 3.5.2 Feeding studies

Since there are no indications from additional studies with the MON 863 X MON 810 hybrid that the genetic modifications of MON 863 and MON 810 mutually affect each other, the results from feeding studies using MON 863 and MON 810 can also be applied to the safety assessment of the MON 863 X MON 810 hybrid.

#### 3.5.2.1 Feeding studies using MON 863 maize kernels

In a **subchronic feeding study with Sprague–Dawley rats,** given feed containing genetically modified MON 863 maize kernels, with proportions of 11 percent and 33 percent in the feed, over an exposure interval of 90 days (20 male and 20 female animals for each dose), no substance-specific biologically relevant effects were seen in comparison to controls reviewing the non-transgenic hybrid or six additional, non-transgenic commercial hybrids. Clinical parameters of haematology, clinical chemistry and urine chemistry were investigated, body weight and organ weight measurements were made, and feed consumption and mortality were determined. From this extensive study, it can be deduced that even after long-term oral exposure to MON 863 maize kernels, no harmful effects are to be expected.

The result of a 42-day **feeding experiment with chickens** (50 each male and female animals), whose feed contained as much as approximately 60 percent genetically modified MON 863 maize, did not yield any indication that the nutritional properties of the maize were altered by the genetic modification. In a comparison of MON 863 maize with the non-transgenic control (parent line) and six commercial hybrids, no biologically relevant changes were observed with respect to mortality, body weight, cadaver parameters (weights of individual body parts, such as thighs, drumsticks, breast and wings) or meat analyses (water content, protein, breast fat and thigh fat) between chickens that received feed with a proportion of MON 863 maize and chickens that received feed with a proportion of the non-transgenic comparison line or the other six reference lines.

#### 3.5.5.2 Feeding studies using MON 810 maize kernels

In a **subchronic feeding study with Sprague–Dawley rats,** given feed containing genetically modified MON 810 maize kernels, with proportions of 11 percent and 33 percent in the feed, over an exposure interval of 90 days (20 male and 20 female animals for each dose), no substance-specific biologically relevant effects were seen in comparison with the non-transgenic control hybrid and six additional, non-transgenic commercial hybrids. Clinical parameters of haematology, clinical chemistry and urine chemistry were investigated, body weight and organ weight measurements were made, and feed consumption and mortality were determined. From this extensive study, it can be deduced that even with long-term oral exposure to MON 810 maize kernels, no harmful effects are to be expected.

### 3.5.3 Assessment of a possible change in composition

In general, when genetic material is inserted into the genome of a recipient cell, there is a possibility of affecting the expression of genes at the insertion site or genes in genetically linked locations, which can cause changes to the composition of the genetically modified organism as the result of effects on metabolic processes. However, such context alterations can also occur under natural conditions (e.g., by transposition or recombination) and as a consequence of mutagenesis of plant material (e.g., by UV light, gamma radiation and chemical mutagenesis).

#### 3.5.3.1 Compositional Analysis

Using material from four field trials in the USA (1999) and four field trials in Argentina (1999/2000), compositional analyses were performed using the genetically modified maize line MON 863 and the hybrid MON 863 X MON 810 as compared with isogenic maize lines that have not been genetically modified and commercial hybrids. The following parameters were investigated: proximates (protein, fat, ash, moisture), ADF, NDF, amino acids (18), fatty acids (16:0, 18:0, 18:2, 18:3, 20:0, 20:1 and 22:0), vitamins B1, B2, E and folic acid, minerals (Ca, Cu, Fe, Mg, Mn, P, K, Na, Zn), phytic acid and trypsin inhibitor, as well as ferulic acid, inositol, raffinose, *p*-coumaric acid, furfural in the kernels and proximates and ADF and NDF in the leaves. The carbohydrate content of the leaves and kernels was calculated using the measurement results for the proximates.

In these investigations, statistically significant deviations from the controls ($p = 0.05$) were seen with some of the parameters. Almost all of the observed deviations fall within the 99-percent confidence range of the values determined for the commercial varieties, or they lie within ranges for the parameters in question that are known from the literature or correspond to the ranges of other conventional varieties ('historic controls') that have been investigated in earlier Monsanto studies. The exceptions to this situation consist of the values for vitamin B1 at two locations, one fatty acid (22:0 belenic acid), folic acid, ferulic acid and *p*-coumaric acid at one location, which were slightly outside the range of the possible comparison values, although for some of these parameters, no values were available in the literature and/or no historical controls were available. The observed deviations were not consistently found at all locations and are assessed as being not biologically relevant.

Based on the results of the compositional analysis, it can be concluded that the maize kernels of the transgenic plants are substantially equivalent to conventional maize kernels, with the exception of the newly expressed proteins.

#### 3.5.3.2 Microbial metabolites

A fundamentally different colonisation of the maize plants by microorganisms as the result of the genetic modifications or the associated occurrence of new microbial metabolic products in the plants, is not to be expected. However, there is a possibility that in the case of the MON 863 X MON 810 hybrid, there could be a reduction in fusarium infestation (fusarium ear rot) during cultivation, and thus a lower proportion of fungal toxins (fumonisin B , DON and ZON) in the harvested kernels, depending on the intensity of infestation by European corn borers (Munkvold *et al.*, 1997 & 1999; Valenta *et al.*, 2001). A reduced number of entry portals as the result of reduced feeding damage and the absence of borer larvae acting as vectors for the infiltration of fungal spores into plant tissues are discussed as the cause for the reduced fusarium infestation of the maize ears.

For MON 863 and MON 810, as well as for the MON 863 X MON 810 hybrid, it can therefore be concluded that based on the expected reduction in entry portals for phytopathogenic microorganisms, at least a higher degree of colonisation will not occur. To this extent, a higher concentration of harmful microbial products in the maize kernels of the GMO, in comparison with conventional maize, is not to be expected.

### 3.5.4 Horizontal gene transfer in the digestive tract

Dissemination of the *B.t.* toxin gene and the *npt*II gene integrated into the MON 863 and MON 863 X MON 810 lines from the genetically modified maize to microorganisms of the digestive tract is not to be expected, due to the absence of a selective advantage. In Germany, the *npt*II gene present in MON 863 has been classified as a Group I gene by the *Zentrale Kommission für die Biologische Sicherheit* (ZKBS) [Central Commission for Biological Safety] in the *Stellungnahme der ZKBS zur biologischen Sicherheit von Antibiotika-Resistenzgenen im Genom gentechnisch veränderter Pflanzen* ['Report of the ZKBS on the Biological Safety of Antibiotic Resistance Genes in the Genome of Genetically modified Plants'], published in 1999. Group 1 is described as follows: 'Group 1 consists of antibiotic resistance genes that (a) are already widely distributed in soil bacteria and enterobacteria and (b) whose relevant antibiotics are of little or no therapeutic significance for human or veterinary medicine, so that it can be concluded that the presence of such an antibiotic-resistance gene (if at all) in the genome of transgenic plants will not have any consequential effect on the dissemination of this antibiotic resistance gene in the environment.' The possibility of transferring the *npt*II gene to microorganisms of the digestive tract was assessed prior to the introduction of the Flavr-Savr tomato in the USA and categorised as harmless. In the past, the ZKBS has agreed with this assessment.

The assumption that there is a low probability of horizontal gene transfer in the digestive tract is supported by investigations in which the DNA of the M13 phage was orally administered to mice, in which phage DNA could only be detected in the faeces for at most 7 hours after feeding. These investigations did not produce any indications of colonisation of the intestinal flora by bacteria containing foreign DNA. Very small quantities of foreign DNA (< 0.1 %) could be identified in the bloodstream for a short time (at most 24 hours) (Schubbert *et al.*, 1994).

With regard to the use of the genetically modified maize for the production of animal feed, it can thus be concluded that harmful effects to the health of animals as the result of horizontal gene transfer are not to be anticipated.

## 3.6 Estimation of risks to the environment

The cultivation of the genetically modified maize in the EU is presently neither registered nor the subject of the application, so it can be concluded that genetically modified maize plants arising from the line MON 863 or the MON 863 X MON 810 hybrid can find their way into the environment only unintentionally and in small quantities.

### 3.6.1 Assessment of the ability of the genetically modified maize to survive or become established and the possibility of transferring the introduced gene to other plants via pollen

Maize is an example of a highly domesticated type of plant, which means that since among other things it does not shed its seeds, it can only survive by human cultivation. Furthermore,

maize plants are not winter hardy, and under the climatic conditions present in Europe, they cannot establish themselves in the natural flora.

Crossing with native wild plants is not possible, since there are not any suitable crossing partners for maize in the Central European flora. The introduced gene can thus only be transferred by means of pollen transfer to the plants of other fields of maize. As a rule, harvested maize is not used as seed for subsequent crops, so the new properties will not spread into cultivated maize varieties via this path.

It is not to be anticipated that the properties of maize described above are affected by the genetic modifications described in the application. In field trials with genetically modified plants, the applicant has carried out studies of the various compositions, vegetative development, flowering, seed ripening and the behaviour of the plants with respect to diseases, as well as yield. In these tests, it was confirmed that with regard to the stated properties, the genetically modified plants do not significantly differ from maize lines that have not been genetically modified. In the case of genetically modified maize plants that are unintentionally released, the possibilities of survival, dissemination, establishment and pollen transfer can only be assessed in the same way as for traditionally cultivated maize.

### 3.6.2 Assessment of environmental changes induced by the transferred gene

Within the context of the introduction of the maize forming the subject of the present application, no threat to the environment is to be expected, due to the mode of action of the Cry protein and NPT II that are formed within the genetically modified plants.

#### 3.6.2.1 *MON 863 cry3Bb1* and *cry1A(b)*

Both of these genes code for protein toxins. There are no indications of enzymatic activity of the proteins expressed in the GMO. It can consequently be concluded that aside from the formation of Cry toxins in the genetically modified plants, there will not be any other effect on metabolism. This assumption is based in particular on the results of the compositional analysis. Furthermore, the assessment of the agronomic parameters and the phenotypic characterisation of the GMO do not yield any recognisable effects on vegetative development and metabolism resulting from expression of the Cry toxins.

Should it happen that small numbers of genetically modified maize plants find their way into the environment, a lasting harmful effect on the environment would not be anticipated, due to the selective mode of action of Cry toxins, including specific receptor binding in the intestinal tracts of sensitive insects.

#### 3.6.2.2 *nptII*

More specific information regarding the characterisation and mode of action of NPT II (aminoglycoside 3' phosphotransferase type II, or APH(3')II) and the potential effects of a horizontal gene transfer are provided in other places in this assessment report.

### 3.6.3 Assessment of horizontal gene transfer from the GMO to microorganisms

Horizontal gene transfer from plants to environmental microorganisms cannot be excluded, although it can be classified as improbable, based, among other reasons, on the fact that the maize forming the subject of the present application is not to be cultivated in Europe.

Effects due to such a gene transfer could only be expected in the presence of a selection pressure favouring the transferred gene. Furthermore, in performing the assessment, consideration must be given to whether the gene in question is already present in the relevant populations or is a new gene.

The *MON 863 cryBb1* gene was derived from the naturally occurring *cry3Bb1* gene from *Bacillus thuringiensis* ssp. *kumamotoensis* and optimised with regard to toxicity to the target organisms. The *cry1A(b)* gene was derived from the naturally occurring cry1A(b) gene from *Bacillus thuringiensis* ssp. *kurstaki* and optimised for expression in plants. *Bacillus thuringiensis* strains containing such δ-endotoxin genes are frequently present on plants, as well as in animal feeds. Consequently, there is a high probability that these genes could also find their way into intestinal microorganisms, or into the environment, by horizontal transfer from *Bacillus thuringiensis* strains that have not been genetically modified. There would not be any associated selection advantage.

For an assessment of the possible effects of a horizontal gene transfer of *npt*II to environmental microorganisms, the statements made above are also applicable. Due to the widespread distribution of kanamycin resistance and neomycin resistance in environmental organisms and the absence of selection pressure, no harmful effects are to be anticipated in the unlikely case of a horizontal transfer of the *npt*II gene.

The remainder of the genetic elements introduced into the genetically modified maize originate from rice, wheat, CaMV, *Tn5* and *Agrobacterium tumefaciens*. They are already frequently present in the environment, so there is a high probability that they could also find their way into microorganisms in the environment via horizontal gene transfer from organisms that have not been genetically modified.

## 4 Monitoring plan

The objectives of the monitoring plan are (i) to confirm that any assumptions regarding the occurrence and impact of potential adverse effects of a GMO and its use made in the environmental risk assessment (ERA) ('case-specific monitoring') are correct, and (ii) to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not foreseen in the ERA ('general surveillance') (see Directive 2001/18/EC, Annex VII).

(i) From the results of the ERA, in which MON 863 and MON 863 X MON 810 were compared with conventional maize with regard to persistence and invasiveness, selection advantage, the potential for gene transfer, effects on non-target and target organisms, effects on biogeochemical processes and modifications to agricultural practice, it can be concluded that the probability of the occurrence of adverse environmental effects (risk) due to the import of the genetically modified maize kernels is negligible. In comparison with conventional maize, no additional effects are to be expected from the genetically modified maize. This applies to import and processing, including use as animal feeds. For this reason, case-specific monitoring is not necessary.

(ii) With regard to general surveillance, in accordance with the intended purposes as stated in the application (import, processing and use as animal feeds), the applicant plans to do the following:

- make product information for MON 863 and MON 863 X MON 810 available to traders and the processing industry, and request these parties to inform the responsible authorities of any adverse effects of the GMO with regard to human health and the environment,

- directly inform the European animal feed industry of the introduction of MON 863 and MON 863 X MON 810, by means of a public announcement,
- request parties active in the animal feed chain to forward to the responsible authorities any reports of adverse effects on animal health arising in connection with MON 863 or MON 863 X MON 810 that are reported to them by farmers or animal-feed associations,
- during the entire term of validity of the registration, immediately inform the EU Commission and responsible national authorities if reports of adverse effects of MON 863 or MON 863 X MON 810 become known.

The RKI is of the opinion that in addition to the above, the national veterinary services of each country (such as veterinarians, veterinary medicine services and associations) and national agencies for animal nutrition and animal feed research (such as the *Bundesforschungsanstalt für Landwirtschaft* in Germany) should also be informed of the introduction of MON 863 and MON 863 X MON 810.

The applicant is to annually submit a report to the RKI regarding the results of the monitoring.

# 5 Conclusions and Conditions

## 5.1 Conclusions

The documents that were submitted by Monsanto included the information necessary for a safety assessment. The examination and assessment of the contents of the documents and the environmental impact assessment have led to the conclusion that no adverse effects to human health or the environment are to be expected from the placing on the market of the genetically modified maize. Based on the current state of knowledge, the RKI concludes that products made from the maize forming the subject of the present application are just as safe as products made from conventional maize.

Based on investigations of chemical composition and the agronomic, morphological, phenotypic, nutritional and toxicological characterisation of the GMO, a substantial equivalence, with the exception of the new characteristics, can be concluded as a starting point for the safety assessment. Conventional maize, for which there is experience with many years of cultivation and use as a raw material for foods and feeds, was used as the reference for comparison.

Since the objective of the genetic modification is to improve agronomic characteristics, no effect on the further use of this maize as a raw material for producing feeds is expected with regard to processing methods and quantitative usage.

The molecular analysis of the genetically modified plants provides comprehensive data regarding the genetic modification that has been performed. This data does not provide any indications that adverse effects are to be expected.

The applicant has presented suitable information regarding assay and identification methods.

A monitoring plan has been presented. Due to the absence of indications of possible hazards, no case-specific monitoring is planned.

## 5.2 Conditions and reservations regarding licensing by the Robert Koch Institut

In order to avoid anticipating the result of the debate at the European level regarding the handling of applications for the registration of genetically modified organisms containing specific genes for antibiotic resistance, the conditions under which the RKI will register the GMO in question, which contains the *npt*II gene, are made subject to the results of the current discussions regarding Art. 4, Sect. 2 of Directive 2001/18/EC.

As a consequence of Art. 4, Sect. 2, there will be a stepwise phasing-out before 31/12/2004 of antibiotic resistance markers that convey resistance against antibiotics used in medical or veterinary treatments and that may have adverse effects on human health or the environment. The responsible body of the Commission has established a working group to develop a concept for assessing antibiotic resistance markers (Working Group on Antibiotic Resistance Marker Genes – Art. 4 (2) of Directive 2001/18/EC, 1st meeting, 2 April 2004). The RKI intends to grant a registration of maize kernels of the MON 863 line and the MON 863 X MON 810 hybrid in accordance with the considerations of this working group or the responsible body of the Commission. If at the time that the decision is to be taken, a common position has not yet been achieved that confirms the assessment of the RKI that no adverse effects on human health or the environment are to be expected from the presence of the *npt*II gene in the GMO forming the subject of the present application, the RKI will consider limiting the term of the registration to 31 December 2004, depending on the status of the discussion.

The RKI intends to impose conditions on the registration to the effect that new regulations regarding labelling and traceability will also be applied to the GMO forming the subject of the present application.

In addition to the proposed monitoring plan, the RKI considers it to be necessary to inform the responsible members of the veterinary profession within the Member States, as well as national authorities responsible for animal nutrition and animal feed research, of the introduction of the GMO, and to include them in the general surveillance programme. Furthermore, annual reports on the results of the monitoring are required.

Corresponding conditions are to be incorporated into the provisions of the authorization.

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