Application for authorization to place on the market LY038 × MON 810 maize in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed

Part II

Summary

A. GENERAL INFORMATION

1. Details of application

a) Member State of application

The Netherlands.

b) Notification number

Not known at the time of application.

c) Name of the product (commercial and other names)

The Renessen LLC development code for this genetically modified maize is: LY038 × MON 810. In countries where LY038 × MON 810 maize* will be cultivated, hybrid seeds of this maize will be marketed in association with the MAVERA trademark, and it will be indicated to growers that those hybrids have increased lysine content and are protected against specific lepidopteran insect pests.

d) Date of acknowledgement of notification

Not known at the time of application.

2. Applicant

a) Name of applicant

Renessen LLC, represented by Renessen Europe SPRL.

b) Address of applicant

Renessen Europe SPRL
Avenue de Tervuren 270
B-1150 Brussels
Belgium

Renessen LLC
520 Lake Cook Road, Suite 220
Deerfield, Illinois 60015
U.S.A.

c) Name and address of the person established in the Community who is responsible for the placing on the market, whether it be the manufacturer, the importer or the distributor, if different from the applicant (Commission Decision 2004/204/EC Art 3(a)(ii))

LY038 × MON 810 maize is produced in other world areas and will be imported and used in the European Union by operators that have traditionally been involved in the commerce, transport, processing and use of maize and maize-derived products in the European Union.

* Hereafter referred to as LY038 × MON 810.
MAVERA is a trademark of Renessen LLC.

3. Scope of the application

(x) GM plants for food use
(x) Food containing or consisting of GM plants
(x) Food produced from GM plants or containing ingredients produced from GM plants
(x) GM plants for feed use
(x) Feed containing or consisting of GM plants
(x) Feed produced from GM plants
(x) Import and processing (Part C of Directive 2001/18/EC)
( ) Seeds and plant propagating material for cultivation in Europe (Part C of Directive 2001/18/EC)

4. Is the product being simultaneously notified within the framework of another regulation (e.g. Seed legislation)?

Yes ( )	**No (x)**
If *yes*, specify	

5. Has the GM plant been notified under Part B of Directive 2001/18/EC and/or Directive 90/220/EEC?

Yes ( )	**No (x)**
If *no*, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC The protein expression, composition, safety, agronomic and phenotypic characteristics of LY038 × MON 810 have been studied at multiple locations in North and South America that cover a range of environmental conditions. The data collected from these field releases have been used in the risk assessment presented in the LY038 × MON 810 application. A summary of the conclusions of the risk analysis that demonstrate the safety of LY038 × MON 810 to humans, animals and to the environment have been presented in the respective sections throughout this summary.	

6. Has the GM plant or derived products been previously notified for marketing in the Community under Part C of Directive 2001/18/EC or Regulation (EC) 258/97?

Yes ( )	**No (x)**
If *yes*, specify	

7. Has the product been notified in a third country either previously or simultaneously?

Yes (x)	No ( )

If *yes*, specify

LY038 × MON 810 has been notified in Argentina for the full range of uses as traditional maize, including the cultivation of varieties. In addition, applications for the import and use of LY038 × MON 810 are currently under review in other countries around the world. The scope and the status of these pending regulatory reviews typically depend on the country and its local regulatory framework.

8. General description of the product

a) Name of the recipient or parental plant and the intended function of the genetic modification

LY038 × MON 810 is traditionally bred maize produced by the cross of two inbred lines, one derived from LY038 and the other from MON 810. Whereas LY038 × MON 810 results from traditional breeding, genetic modification was used in the development of the single-trait, parental inbred lines LY038 and MON 810.

LY038 produces the *Corynebacterium glutamicum*-derived lysine-insensitive dihydrodipicolinate synthase (cDHDPS) protein. Compared with traditional maize, LY038 grains have increased lysine content and improved nutritional value for use as a feed ingredient in animal diets, primarily for broilers, turkeys and swine.

MON 810 produces the Cry1Ab protein derived from *Bacillus thuringiensis* subsp. *kurstaki* (strain HD-1), which confers protection against certain lepidopteran insect pests, including the European Corn Borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp.).

As LY038 × MON 810 inherits the introduced traits from its parental single-trait maize inbreds, this maize has higher lysine content grain and is protected against the targeted lepidopteran insect pests.

b) Types of products planned to be placed on the market according to the authorisation applied for

This application is for authorization of LY038 × MON 810 maize for import, food and feed use according to Articles 5 and 17 of Regulation (EC) No 1829/2003 of 22 September 2003 on genetically modified food and feed. It should be noted that LY038 × MON 810 is only intended for use as a feed, but in the unlikely event that LY038 × MON 810 is inadvertently used as food, the scope of the safety assessment covers both feed and food uses. The scope of this application does not include the cultivation of LY038 × MON 810 varieties in the EU.

c) Intended use of the product and types of users

LY038 × MON 810 will be a value-added specialty animal feed crop. LY038 × MON 810 will be initially cultivated in Argentina and the U.S.A. It is planned that specific lots of LY038 × MON 810 will be sold per commercial or customer specifications and transported as a specialty crop from the production field, where LY038 × MON 810 is grown, to specific end customers (feed manufactures and livestock producers) in the EU.

d) Specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for

No specific conditions are considered necessary for the placing on the market of LY038 × MON 810. LY038 × MON 810 maize is substantially equivalent to traditional maize, except for the introduced lysine trait and for the protection against certain lepidopteran insect pests. This maize was shown to be as safe as traditional maize. Therefore, grain, forage and products produced from LY038 × MON 810 will be stored, packaged, transported, handled and used in the same manner as traditional maize products, with the exception of those practices needed to retain the commercial value of the increased lysine in this product.

e) Any proposed packaging requirements

LY038 × MON 810 maize is substantially equivalent to traditional maize, except for the introduced lysine trait and for the protection from certain lepidopteran insect pests. Therefore, LY038 × MON 810 and derived products will be used in the same manner as other maize and no specific packaging is required. (For the labeling, *see* question 8.(f)).

f) Any proposed labelling requirements in addition to those required by Community law (Annex IV of Directive 2001/18/EC; Regulation 1829/2003 art. 13 and 25)

In accordance with Regulations (EC) No 1829/2003 and 1830/2003, a labeling threshold of 0.9% is applied for the placing on the market of LY038 × MON 810 and derived products.

Operators shall be required to label products containing or consisting of LY038 × MON 810 with the words "genetically modified maize" or "contains genetically modified maize", and shall be required to declare the unique identifier REN-ØØØ38-3 × MON-ØØ81Ø-6 in the list of GMOs that have been used to constitute a mixture that contains or consists of this GMO.

Operators shall be required to label feeds derived from LY038 × MON 810 with the words "produced from genetically modified maize". In the case of products for which no list of ingredients exists, operators shall ensure that an indication that the feed product is produced from GMOs is transmitted in writing to the operator receiving the product.

Operators handling or using LY038 × MON 810 and derived feeds in the EU are required to be aware of the legal obligations regarding

traceability and labeling of these products. Given that explicit requirements for the traceability and labeling of GMOs and derived foods, and feeds are laid down in Regulation (EC) No 1829/2003 and 1830/2003, and that authorized foods and feeds shall be entered in the Community Register, operators in the food/feed chain will be fully aware of the traceability and labeling requirements for LY038 × MON 810. Therefore, no further specific measures are to be taken by the applicant.

g) Unique identifier for the GM plant (Regulation (EC) 65/2004; does not apply to applications concerning only food and feed produced from GM plants, or containing ingredients produced from GM plants)

REN-ØØØ38-3 × MON-ØØ81Ø-6.

LY038 × MON 810 is uniquely identified using the combination of the unique identifiers for LY038 (REN-ØØØ38-3) and MON 810 (MON-ØØ81Ø-6).

h) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for. Any type of environment to which the product is unsuited

LY038 × MON 810 is suitable for use throughout the EU.

9. Measures suggested by the applicant to take in case of unintended release or misuse as well as measures for disposal and treatment

Because this application is for consent to import and use LY038 × MON 810 as any other maize, not including the cultivation of LY038 × MON 810 varieties in the EU, any environmental release would be more likely to occur during import, storage and processing of LY038 × MON 810. However, LY038 × MON 810 is designed as a value-added specialty animal feed crop, which will attract a premium price when compared with traditional field maize. Therefore, operators in the supply chain will have a major incentive to establish procedures that minimize loss of LY038 × MON 810 grain from production to consumption. LY038 × MON 810 grain will be held, transported and handled in a confined manner that will significantly limit the entry of LY038 × MON 810 grain into to the environment. In the event of incidental spillage, the establishment of volunteer plants would be unlikely, since maize cannot survive without human assistance and is not capable of surviving as a weed. Volunteer maize is not found growing in fencerows, ditches, and roadsides. Maize volunteers, if they occurred, would be likely to be killed by frost or could be easily controlled by the use of selective herbicides.

The information presented in this application establishes that LY038 × MON 810 is not different from other maize and, therefore, is unlikely to pose any threat to the environment or to require special measures for its containment.

In conclusion, no specific measures to manage incidental unintended release or misuse are considered necessary.

B. INFORMATION RELATING TO (A) THE RECIPIENT OR (B) (WHERE APPROPRIATE) PARENTAL PLANTS

1. Complete name

a)	**Family name** Poaceae (formerly Gramineae)
b)	**Genus** *Zea*
c)	**Species** *mays* (2n=20)
d)	**Subspecies** *mays*
e)	**Cultivar/breeding line** H99
f)	**Common name** Maize; Corn

2. a) Information concerning reproduction 10

(i) Mode(s) of reproduction Maize (*Zea mays* L.) is an annual, wind-pollinated, monoecious species with separate staminate (tassels) and pistillate (silk) flowers. Self- and cross-pollination are generally possible, with frequencies of each normally determined by proximity and other physical influences on pollen transfer.
(ii) Specific factors affecting reproduction Tasselling, silking, and pollination are the most critical stages of maize development and, consequently, grain yield may ultimately be greatly impacted by moisture and fertility stress.
(iii) Generation time Maize is an annual crop with a cultural cycle ranging from as short as 60 to 70 days to as long as 43 to 48 weeks from seedling emergence to maturity.

2. b) Sexual compatibility with other cultivated or wild plant species

Out-crossing with cultivated *Zea* varieties

The scope of the current application does not include cultivation of LY038 × MON 810 varieties in the EU. Outcrossing with cultivated *Zea* varieties is therefore not expected.

Out-crossing with wild *Zea* species

There are no wild relatives of maize in Europe.

3. Survivability

a) Ability to form structures for survival or dormancy

Maize is an annual crop and seeds are the only survival structures. Natural regeneration from vegetative tissue is not known to occur.

b) Specific factors affecting survivability

Maize cannot survive without human assistance and is not capable of surviving as a weed due to past selection in its domestication. Volunteer maize is not found growing in fencerows, ditches or roadsides as a weed. Although maize seed from the previous crop year can survive in mild winter conditions and germinate the following year, it cannot persist as a weed. The appearance of "volunteer" maize in fields following a maize crop from the previous year is rare under European conditions. Maize volunteers are killed by frost or, in the unlikely event of their occurrence, are easily controlled by current agronomic practices including soil cultivation practices and the use of selective herbicides.

Maize grain survival is dependent upon temperature, moisture of seed, genotype, husk protection and stage of development. Freezing temperatures have an adverse effect on maize seed germination and have been identified as being a major risk in seed maize production. Temperatures above 45°C have also been reported as injurious to maize seed viability.

4. Dissemination

a) Ways and extent of dissemination

Dissemination of maize may occur by means of seed dispersal and pollen dispersal. Dispersal of the maize grain is highly restricted in domesticated maize due to the ear structure including husk enclosure. For maize pollen, the vast majority is deposited in the same field due to its large size (90 to 100 µm) with smaller amounts of pollen deposited usually in a downwind direction.

b) Specific factors affecting dissemination

Dispersal of maize seeds does not occur naturally because of the structure of the ears of maize. Dissemination of isolated seeds may result from mechanical harvesting and transport as well as insect or wind damage, but this form of dissemination is highly infrequent. Genetic material can be disseminated by pollen dispersal, which is influenced by wind and weather conditions. Maize pollen is the largest of any pollen normally disseminated by wind from a comparably low level of elevation. Dispersal of maize pollen is limited by its large size and rapid settling rate.

5. Geographical distribution and cultivation of the plant, including the distribution in Europe of the compatible species

Because of its many divergent types, maize is grown over a wide range of climatic conditions. The bulk of the maize is produced between latitudes 30° and 55°, with relatively little grown at latitudes higher than 47° latitude anywhere in the world. The greatest maize production occurs where the warmest month isotherms range between 21° and 27°C and the freeze-free season lasts 120 to 180 days. A summer rainfall of 15 cm is approximately the lower limit for maize production without irrigation with no upper limit of rainfall for growing maize, although excess rainfall will decrease yields.

There are no wild relatives of maize in Europe.

6. In the case of plant species not normally grown in the Member State(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts

Maize is widely grown in the European Union. The most important areas of maize production in Europe include the Danube Basin, from southwest Germany to the Black Sea, along with southern France through the Po Valley of northern Italy.

7. Other potential interactions, relevant to the GM plant, of the plant with organisms in the ecosystem where it is usually grown, or used elsewhere, including information on toxic effects on humans, animals and other organisms

Maize is known to interact with other organisms in the environment including insects, birds, and mammals. It is susceptible to a range of fungal diseases and nematode, insect and mite pests. Maize has a history of safe use for human food and animal feed.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification

No novel method of genetic modification is utilized in the production of LY038 × MON 810. Instead, LY038 × MON 810 is traditionally bred maize produced by the cross of two inbred lines, one derived from LY038 and the other from MON 810. While LY038 × MON 810 results from traditional breeding, genetic modification was used in the development of the single-trait, parental lines LY038 and MON 810.

2. Nature and source of the vector used

LY038 × MON 810 was produced by crossing single-trait inbred plants of LY038 and MON 810 using traditional breeding methods. No vector has been used to generate this maize hybrid. Instead, the single-trait parental lines LY038 and MON 810 were both obtained by genetic modification. LY038 was produced by the particle acceleration method using a 5.9 kb DNA fragment derived from plasmid vector PV-ZMPQ76, containing a dihydrodipicolinate synthase (DHDPS) gene originating from the common soil bacterium *Corynebacterium glutamicum* (cDHDPS). MON 810 was generated by the integration of sequences from the plasmid vector PV-ZMBK07, containing the *cry1Ab* coding sequence of interest, which was derived from *Bacillus thuringiensis* subsp. *kurstaki*.

3. Size, source (name) of donor organism(s) and intended function of each constituent fragment of the region intended for insertion

LY038 × MON 810 is traditionally bred maize produced by the cross of two inbred lines, one derived from LY038 and the other from MON 810. LY038 × MON 810 hybrid seed (F1) inherits the LY038 and MON 810 inserts from its parental inbred lines. The individual components of the inserts and the function of these inherited DNA sequences are given in Table 1 and 2.

Table 1. Components of the LY038 insert

Genetic Element	Source	Size (kb)	Function
Glb1	*Zea mays* L.	1.4	The promoter from the *Globulin* 1 (Glb1) gene, which in wild-type maize directs expression of globulin, the most abundant embryo-specific protein in maize grain
rAct1 intron	*Oryza sativa* L.	0.5	The intron sequence derived from the rice actin-1 gene to enhance DNA transcription
mDHDPS CTP	*Zea mays* L.	0.2	The chloroplast targeting sequence from dihydrodipicolinate synthase (DHDPS)
cordapA	*Corynebacterium glutamicum*	0.9	The coding sequence from the dihydrodipicolinate synthase (*dapA*) in the lysine biosynthetic pathway, conferring resistance to lysine feedback inhibition
Glb1 3' UTR	*Zea mays* L.	1	The 3' non-translated region from the *Globulin* 1 (Glb1) gene which directs the polyadenylation of the mRNA

Table 2. Components of the MON 810 insert

Genetic Element	Source	Size (kb)	Function
CaMV e35S	Cauliflower mosaic virus	0.32	Promoter
Zm.hsp70	*Zea mays* L.	0.8	Stabilizes the level of gene transcription.
cry1Ab	*Bacillus thuringiensis*	3.5	Encodes the Cry1Ab protein, which targets specific lepidopteran insect pests

D. INFORMATION RELATING TO THE GM PLANT

1. Description of the trait(s) and characteristics which have been introduced or modified

LY038 × MON 810 was produced by crossing single-trait inbred plants of LY038 and MON 810 using traditional breeding methods. LY038 × MON 810 therefore expresses the cDHDPS protein, which determines the increase of lysine content in maize grain and the Cry1Ab protein, which confers protection against certain lepidopteran insect pests.

Like LY038, LY038 × MON 810 contains the *cordapA* gene encoding a dihydrodipicolinate synthase from *Corynebacterium glutamicum* (cDHDPS), which determines the increase of lysine content in LY038 × MON 810 maize grain. Dihydrodipicolinate synthase (DHDPS), the first enzyme in lysine biosynthesis, mediates a critical rate-limiting step in the lysine biosynthetic pathway. The enzyme catalyzes the condensation of L-aspartate-4-semialdehyde and pyruvate to form 2,3-dihydrodipicolinate, which is subsequently converted to lysine through a series of successive enzymatic reactions. DHDPS is highly susceptible to lysine feedback inhibition. In contrast to the native *Zea mays* DHDPS (mDHDPS) enzyme, the variant of this enzyme encoded by *Corynebacterium glutamicum* (cDHDPS), is much less sensitive to lysine feedback inhibition, resulting in increased accumulation of free lysine in the grain of LY038 × MON 810.

The Cry1Ab protein from MON 810 is also expressed in LY038 × MON 810. This protein was derived from *Bacillus thuringiensis* subsp. *kurstaki* and confers protection against predation by certain lepidopteran insect pests, including the European Corn Borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp). The cultivation of LY038 × MON 810 will enable farmers to effectively control these maize pests, ensuring maximum realization of yield potential, while reducing the environmental burden of the production, packaging, transport and use of insecticides, previously used to control *Ostrinia nubilalis* and *Sesamia* spp.

To conclude, LY038 × MON 810 hybrids inherit the increased lysine content from LY038 and the protection against important insect pests from MON 810.

2. Information on the sequences actually inserted or deleted

a) The copy number of all detectable inserts, both complete and partial

As described in the respective applications for the single-trait parental maize lines, LY038 and MON 810 each contain a single DNA insert with a single copy of the introduced DNA fragment, and this at different loci in the maize genome. In the progeny of LY038 and MON 810, each fragment is inherited as a single gene in a Mendelian fashion.

As the parental maize lines, used to produce LY038 × MON 810 through traditional breeding, are inbred lines that are homozygous for the LY038 or MON 810 inserts, respectively, both of the inserted

fragments are inherited in LY038 × MON 810 hybrid seed, *i.e.* one fragment conferring the increase of lysine and the other imparting protection against certain lepidopteran insect pests. The presence of both inserts in LY038 × MON 810 was confirmed through Southern blot analysis, showing that the integrity of the inserts has been conserved in the combined-trait product.

b) In case of deletion(s), size and function of the deleted region(s)

Not applicable.

c) Chromosomal location(s) of insert(s) (nucleus, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination

LY038 × MON 810 contains both parental inserts on separate chromosomes in the nuclear genome, as they were present in LY038 and MON 810, respectively. The presence of the inserts from LY038 and MON 810 in LY038 × MON 810 was confirmed by Southern blot analyses.

d) The organisation of the inserted genetic material at the insertion site

The molecular comparison of LY038 × MON 810 to the single-trait parental lines, LY038 and MON 810, indicates that the inserts are preserved in LY038 × MON 810. There is no scientific basis to support the fact that those inserts would be intrinsically more unstable when combined together by traditional breeding. The molecular characteristics of the respective introduced DNA sequences, present in the single-trait parental lines LY038 and MON 810, also apply to LY038 × MON 810, including the structural organization and integrity of the inserts, as well as the characteristics of the sites of insertion and the flanking sequences, immediately adjacent to the introduced sequences.

3. Information on the expression of the insert

a) Information on developmental expression of the insert during the life cycle of the plant

As LY038 × MON 810 maize was bred from its parental lines LY038 and MON 810, inheriting respectively the introduced *cordapA* and *cry1Ab* genes, it is expected that the combined-trait product produces the cDHDPS protein, as in LY038, and the Cry1Ab protein, as in MON 810.

The levels of cDHDPS and Cry1Ab were assessed in various maize tissues using validated enzyme-linked immunosorbent assays (ELISA). However, only results for forage and grain tissue samples are presented here, because these tissues are most relevant for the evaluation of the food and feed safety of LY038 × MON 810. Levels of these proteins were also determined in the tissues of the respective single-trait parental

products LY038 and MON 810.

Analytical results were obtained as micrograms (µg) of the specific protein per gram (g) of tissue on a fresh weight (fw) basis and then converted to a dry weight (dw) basis.

The mean cDHDPS level was 27 µg/g dw in LY038 × MON 810 grain samples, as compared to 26 µg/g dw in grain from the single-trait LY038 control. The mean level of the cDHDPS protein in LY038 × MON 810 forage was 0.86 µg/g dw, as compared to the mean level of 0.94 g/g dw in LY038 forage.

In LY038 × MON 810 grain samples, the mean Cry1Ab protein level was 0.43 µg/g dw, as compared to 0.53 µg/g dw in MON 810 grain. The mean Cry1Ab level in LY038 × MON 810 forage was 14 µg/g dw, as compared to 14 µg/g dw in forage from the single-trait MON 810 control.

As expected, considering the limit of quantitation values for each ELISA assay, the cDHDPS and Cry1Ab proteins were not detected in grain from the MON 810 and LY038 plants, respectively.

The presented data establish the expression levels of cDHDPS and Cry1Ab in grain and forage from LY038 × MON 810. These expression levels are consistent with the levels observed in the respective single-trait controls and with levels that have been previously reported for the single-trait products from trials conducted during other production years.

b) Parts of the plant where the insert is expressed

The transcription of the *cordapA* coding sequence is under the control of the maize Glb1 promoter, which directs the cDHDPS expression predominantly in the grain. The production of the Cry1Ab protein occurs throughout the plant since the CaMV e35S promoter has been shown to drive constitutive expression of the encoded protein in genetically modified maize.

4. Information on how the GM plant differs from the recipient plant in

a) Reproduction

Based on centuries of experience with traditional, domesticated maize in the EU, there is negligible potential for maize to be invasive of natural habitats or to persist in the agronomic environment without the aid of human intervention. The maize plant is known as a poor competitor, which, outside of cultivation, has no meaningful impact on the environment.

Agronomic data collected from trials conducted with LY038 × MON 810 have demonstrated that LY038 × MON 810 has not been altered in survival, multiplication and dissemination characteristics when compared to traditional maize.

It is therefore possible to conclude that no differences in the mode or rate of reproduction, maize grain dissemination, survivability or other

phenotypic characteristics are expected in LY038 × MON 810 and that LY038 × MON 810 is equivalent to traditional maize in its reproductive behavior.

b) Dissemination

The introduced traits have no influence on maize reproductive morphology and hence no changes in seed dissemination are to be expected.

c) Survivability

Maize is known to be a weak competitor in the wild, which cannot survive in Europe outside cultivation without the aid of human intervention. Field observations have demonstrated that LY038 × MON 810 has not been altered in its survivability when compared to traditional maize.

d) Other differences

Comparative assessments of the phenotypic characteristics of LY038 × MON 810 and traditional maize in the field did not reveal biologically significant differences.

5. Genetic stability of the insert and phenotypic stability of the GM plant

LY038 × MON 810 hybrid seed (F1) is produced by traditional breeding, starting from LY038 and MON 810 inbred lines (made homozygous for either the LY038 or the MON 810 insert, respectively). Thereby, each parental line passes on its inserted DNA sequence to the resulting LY038 × MON 810 plant.

The single-trait products, LY038 and MON 810, each contain one insert which is stably integrated into the nuclear maize genome. In the progeny of LY038 and MON 810, each introduced trait is inherited as a single dominant gene in a Mendelian fashion. This has been confirmed by Southern blot analyses and by studies of the inheritance pattern of these traits in maize.

The harvested (F2) grain of LY038 × MON 810 is marketed by the grower for feed use and is not used for further breeding. Therefore, since LY038 × MON 810 hybrid maize seed exists only for a single generation, there is no opportunity for its stability to be compromised.

6. Any change to the ability of the GM plant to transfer genetic material to other organisms

a) Plant to bacteria gene transfer

No elements known to be involved in DNA mobility have been included in the inserted DNA fragment. Therefore, in comparison to traditional maize, no changes are to be expected in the ability of the GM plant to exchange genetic material with bacteria.

b) Plant to plant gene transfer

Based on the observation that reproductive morphology in the single-trait products and in the LY038 × MON 810 combined-trait product is unchanged compared to traditional maize, the out-crossing frequency to other maize varieties or to wild relatives (which are not present in the EU) would be unlikely to be different for LY038 × MON 810, when compared to traditional maize varieties.

However, the scope of the current application does not include the cultivation of LY038 × MON 810 varieties in the EU.

7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed

7.1 Comparative assessment

Choice of the comparator

LY038 × MON 810 was compared to a traditional maize hybrid with background genetics similar to LY038 × MON 810, (*i.e.* the control).

7.2 Field trials

a) number of locations, growing seasons, geographical spreading and replicates

LY038 × MON 810, its control and a number of commercial maize hybrids were grown at 5 field sites in the U.S.A. during the 2002 growing season. At each field site, LY038 × MON 810, its control and the commercial maize hybrids were planted in a randomized complete block design with three replicates per plot. The results of the compositional analyses concluded that LY038 × MON 810 is compositionally equivalent to traditional maize, except for the intended increase in lysine and the associated increase in the lysine-related catabolites saccharopine and α-aminoadipic acid in LY038 × MON 810 grain.

b) the baseline used for consideration of natural variations

In the 2002 U.S.A. field trials, four traditionally commercially available maize hybrids were grown at each of the five sites to provide a total of 20 traditional reference hybrids (18 unique

reference hybrids). The commercial maize reference hybrids were analyzed in order to generate data for the development of a 99% tolerance interval for the components tested.

7.3 Selection of compounds for analysis

Both forage and grain samples were collected an analyzed for nutritional components, antinutrients and secondary metabolites in accordance with the recent OECD consensus document on compositional considerations for new varieties of maize. In addition, free lysine and six lysine-related metabolites from the lysine biosynthetic and catabolic pathways were also analyzed in grain.

7.4 Agronomic traits

Compared to traditional maize, LY038 × MON 810 has not been significantly changed with respect to its dispersal or survival characteristics. Further, LY038 × MON 810 is also unchanged compared to traditional maize in terms of invasiveness of natural environments and persistence in the environment, as established by the phenotypic characteristics measured and by the observation of no significant differences in incidence of stressor symptoms, other than the introduced protection of LY038 × MON 810 against certain target lepidopteran insect pests. It is concluded that LY038 × MON 810 does not significantly differ from traditional maize with regard to reproduction, dissemination, survivability and other phenotypic traits.

7.5 Product specification

LY038 × MON 810 comprises all traditionally bred maize, produced by the combination of LY038 and MON 810. As such, LY038 × MON 810 contains both inserts in combination. Therefore, LY038 × MON 810 is detectable using either the event-specific PCR method for detecting the introduced DNA present in LY038, or the equivalent method for MON 810. However, as for all plants in which DNA inserts are combined by traditional breeding, the presence of LY038 × MON 810 may only be confirmed where single kernels are subjected to detection methods for LY038 and MON 810, and test positive for both.

7.6 Effect of the production and processing

Due to its nutritional characteristics, LY038 × MON 810 is destined exclusively for animal feed uses. The effects of production and processing of LY038 × MON 810 are not expected to be any different from the production and processing of the equivalent feed materials originating from traditional maize, since LY038 × MON 810 grains has been shown to be compositionally equivalent to traditional maize, with the exception of the intended increase in lysine and the associated increase in two products of lysine catabolism, saccharopine and α-aminoadipic acid in LY038 × MON 810 grain. In the unlikely case that adventitious, trace amounts of LY038 × MON 810 grain might inadvertently enter the food processing stream, free lysine, saccharopine and α-aminoadipic acid will most likely fractionate into the animal feed components or will be present at concentrations that are lower than those present in other commonly used foods.

7.7 Anticipated intake/extent of use

Due to its nutritional characteristics, LY038 × MON 810 is intended to be used as a value-added specialty animal feed crop. LY038 × MON 810 will be initially cultivated in Argentina and the U.S.A. It is planned that specific lots of LY038 × MON 810 will be sold per commercial or customer specifications and transported as a specialty crop from the production field, where LY038 × MON 810 is grown, to specific end customers (feed manufactures and livestock producers) in the EU. LY038 × MON 810 is expected to replace a small portion from current maize hybrids such that its intake will represent some fraction of the total feed products derived from maize in the EU. Anticipated dietary intake of maize and maize-derived feeds in the EU is not expected to be altered upon commercialization of LY038 × MON 810.

7.8 Toxicology

7.8.1 Safety evaluation of newly expressed proteins

LY038 × MON 810 is traditionally bred maize produced by the cross of two inbred lines, one derived from LY038 and the other from MON 810. Both of the introduced traits in the single-trait parental lines are inherited by the LY038 × MON 810 progeny. This results in the combined expression of the cDHDPS and the Cry1Ab proteins in the same plant. These introduced proteins are present at low levels in the plant and have previously been demonstrated as safe for animal and human health, as part of the safety evaluation of the single-trait products, LY038 and MON 810.

The conclusions of the safety assessment of the cDHDPS and Cry1Ab proteins was based on the following considerations: a) history of safe use, b) no amino acid sequence similarity to known toxins, other than *B.t.* proteins, and no immunologically relevant sequence similarity with known allergens, c) rapid degradation in simulated gastric fluid, and d) no indication of acute toxicity in mice administered the cDHDPS or Cry1Ab proteins by oral gavage.

7.8.2 Testing of new constituents other than proteins

Since maize is known as a common source of food and feed with a centuries-long history of safe use and consumption around the world, and as LY038 × MON 810 was shown to be substantially equivalent to traditional maize (except for the introduced lysine and insect protection traits), no testing of any constituent other than the introduced proteins is indicated.

7.8.3 Information on natural food and feed constituents

LY038 × MON 810 grain and forage are compositionally equivalent to traditional maize grain and forage, with the exception of the intended increase in lysine and the associated increase in two lysine-related catabolites, saccharopine and α-aminoadipic acid, in LY038 × MON 810 grain. Lysine is an

essential amino acid and is a common constituent in plant, animal and human proteins. It is reported that consumption of excess lysine by humans, pigs and rats over prolonged periods is well tolerated, indicating that this amino acid exhibits low toxicity. Both saccharopine and α-aminoadipic acid are natural products of normal lysine catabolism in plants and animals. Therefore, animals and humans are commonly exposed to these compounds in the normal course of endogenous lysine metabolism, as well as from commonly consumed feeds and foods.

7.8.4 Testing of the whole GM food/feed

The compositional data establish that LY038 × MON 810 grain and forage are compositionally equivalent to traditional maize grain and forage with the exception of the intended increase in lysine and the associated increase in two lysine-related catabolites, saccharopine and α-aminoadipic acid, in LY038 × MON 810 grain. The safety of the cDHDPS and Cry1Ab proteins, lysine, saccharopine and α-aminoadipic acid for humans and animals has been demonstrated. In addition, the wholesomeness and safety of LY038 × MON 810 has been confirmed in a 42-day feeding study using broiler chickens.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

Absence of any allergenic potential associated with the introduced cDHDPS and Cry1Ab proteins expressed in LY038 × MON 810 has previously been demonstrated for the single-trait products. cDHDPS and Cry1Ab are present at very low levels in LY038 × MON 810 grains. These proteins were assessed for their potential allergenicity by a variety of tests, including a) whether the genes came from allergenic or non-allergenic sources, b) sequence similarity to known allergens, and c) pepsin stability of the protein in an *in vitro* digestion assay. In all cases, the proteins did not exhibit properties characteristic of allergens.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

As the introduced proteins do not have allergenic potential, it was concluded that the use of LY038 × MON 810 for food or feed does not lead to an increased risk for allergenic reactions compared to the equivalent range of food and feed uses of traditional maize.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

See section D.7.10.2.

7.10.2 Nutritional assessment of GM feed

LY038 × MON 810 will be a value-added specialty animal feed crop. LY038 × MON 810 will be initially cultivated in Argentina and the U.S.A. It is planned that specific lots of LY038 × MON 810 will be sold per commercial or customer specifications and transported as a specialty crop from the production field, where LY038 × MON 810 is grown, to specific end customers (feed manufactures and livestock producers) in the EU.

The nutritional value of LY038 × MON 810 was assessed by a 42-day broiler feeding study. The bioefficacy and bioavailability of the incremental lysine expressed in LY038 × MON 810 grain was demonstrated by performance and carcass measurements of birds receiving a diet formulated with LY038 × MON 810, compared to the performance and carcass measurements for birds fed diets supplemented with crystalline lysine (either the control, whose background genetics is representative of LY038 × MON 810, or traditional reference maize included at the same inclusion rate as LY038 × MON 810).

Enhanced growth, feed efficiency and carcass yield due to the increased level of available lysine in LY038 × MON 810 grain were demonstrated by the observed superior performance of broilers fed a diet containing LY038 × MON 810 grain, as compared to that of broilers fed a diet lacking supplemental crystalline lysine, but otherwise identical in composition with the maize component being either the control or traditional reference maize.

Relatively small changes in growth rate, feed efficiency, and/or carcass measurements as a result of a change in nutritional (nutrient or anti-nutrient) or health status can be detected in the fast growing broiler. No unexpected effects on bird performance or health were observed with the feeding of LY038 × MON 810 grain. Therefore, LY038 × MON 810 maize grain can be considered as safe as traditional maize when fed to poultry and more nutritious than traditional maize due to the increased lysine levels in LY038 × MON 810.

7.11 Post-market monitoring of GM food/feed

There are no intrinsic hazards related to LY038 × MON 810 as no signs of adverse or unanticipated effects have been observed in a number of safety studies, including animal feeding studies using doses of administration that are typical for animal feed applications and that are orders of magnitude above possible inadvertent human consumption levels. The pre-market risk characterization for feed use of LY038 × MON 810 demonstrates that the risks of consumption of

LY038 × MON 810 or its derived products are consistently negligible and no different from the risks associated with the consumption of traditional maize and maize-derived products. As a consequence, specific risk management measures are not indicated.

8. Mechanism of interaction between the GM plant and target organisms (if applicable)

LY038 is not pesticidal to any target organism. The spectrum of target organisms of LY038 × MON 810 is therefore identical to the target organisms of MON 810, which has already been approved for cultivation and use in the EU under Directive 90/220/EEC since 1998.

Any significant interactions of LY038 × MON 810 with its target pest organisms are limited to those countries where the cultivation of this maize has been authorized. However, the cultivation of LY038 × MON 810 in the EU is not within the scope of this application.

9. Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification

9.1 Persistence and invasiveness

As for traditional maize, the likelihood of LY038 × MON 810 spreading in the environment is negligible, as maize is neither persistent nor invasive and these parameters are unaltered in LY038 × MON 810. In the unlikely event of establishment of a LY038 × MON 810 plant, *e.g.* from a grain spilt in the environment, its introduced traits would have negligible consequences for the environment. Hence, the risk of unintentional spreading of LY038 × MON 810 through increased weediness of this maize is negligible.

9.2 Selective advantage or disadvantage

Compared to traditional maize, the presence of the introduced traits in LY038 × MON 810 volunteers would only confer a meaningful advantage where target lepidopteran insect pests are present in high numbers and if no other important factors limiting its establishment in the environment are present. The risk of the insect protection trait in LY038 × MON 810 to be the cause of any competitive advantage or disadvantage impacting the environment is negligible, as maize is unlikely to establish outside cultivation under European conditions.

9.3 Potential for gene transfer

There is no potential for gene transfer from LY038 × MON 810 to wild plant species in the EU and negligible likelihood for gene transfer to other maize crops, as this application is not for consent to cultivate LY038 × MON 810 in the EU. Therefore, the environmental risk of potential gene transfer is negligible.

9.4 Interactions between the GM plant and target organisms

Not applicable. The insecticidal activity against certain lepidopteran insect pests of the Cry1Ab protein expressed in LY038 × MON 810 is only applicable in a cultivation scenario, and the cultivation of LY038 × MON 810 varieties in the EU is not within the scope of this application under Regulation (EC) No 1829/2003. The cDHDPS protein is not pesticidal to any target organism.

9.5 Interactions of the GM plant with non-target organisms

Given the scope of the current application, which does not include the cultivation of LY038 × MON 810 in the EU, the likelihood for direct or indirect interactions of this maize with non-target organisms is considered to be negligible. In addition, the newly expressed traits present a negligible hazard to non-target organisms, even if incidental spillage of LY038 × MON 810 grains during import, storage, transport or use would lead to the short survival of LY038 × MON 810 plants in the environment. As a consequence, there is negligible risk for harmful effects of LY038 × MON 810 on non-target organisms.

9.6 Effects on human health

The likelihood for any adverse effects occurring in humans as a result of their contact with this maize is no different from traditional maize, as LY038 × MON 810 contains the cDHDPS and Cry1Ab proteins, which have negligible potential to cause any toxic or allergenic effects in humans, and increased levels of lysine, saccharopine and α-aminoadipic acid, which are metabolites commonly present in nature with a history of safe use. Therefore, the risk for occupational health effects of this maize is negligible.

9.7 Effects on animal health

The likelihood of potential adverse effects in animals fed on LY038 × MON 810 and in humans, consuming those animals, is negligible. Therefore, the risk of LY038 × MON 810 for the food/feed chain is also negligible.

9.8 Effects on biogeochemical processes

In the event of an incidental release of LY038 × MON 810 in the environment, the risk for direct or indirect, immediate or delayed adverse effects on biogeochemical processes can be considered as negligible. There is no evidence that LY038 × MON 810 plants would be any different from traditional maize regarding their direct influence on biogeochemical processes or nutrient levels in the soil, as LY038 × MON 810 has equivalent growth and development, morphology, yield, plant health and survival characteristics to traditional maize. Furthermore, any indirect interactions of the GMO and target or non-target organisms in the vicinity of an incidental release of the grain are not likely to cause hazardous effects on the biogeochemical processes in the soil. As previously discussed, cDHDPS, Cry1Ab, lysine, saccharopine and α-aminoadipic acid are widely present in the environment.

9.9 Impacts of the specific cultivation, management and harvesting techniques

Not applicable. This application is for consent to import LY038 × MON 810 in the EU and for the use of this maize as any other maize, excluding the cultivation of varieties in the EU.

10. Potential interactions with the abiotic environment

LY038 × MON 810 is substantially equivalent to traditional maize, with the exception of the two introduced (*i.e.* inherited) traits, which are imparted by the expression of the cDHDPS and Cry1Ab proteins. cDHDPS and Cry1Ab have a safe history of use and have no known negative interactions with the abiotic environment.

11. Environmental monitoring plan (not if application concerns only food and feed produced from GM plants, or containing ingredients produced from GM plants)

11.1 General (risk assessment, background information)

As required by Article 5(5)(b) of Regulation (EC) No 1829/2003, a general surveillance plan in accordance to Annex VII of Directive 2001/18/EC is included.

11.2 Interplay between environmental risk assessment and monitoring

An environmental risk assessment (e.r.a.) of LY038 × MON 810 was undertaken as required by Articles 5(5) and 17(5) of Regulation (EC) No 1829/2003. Analysis of the characteristics of LY038 × MON 810 has shown that the risk for potential adverse effects on human health and the receiving environment, resulting from the proposed use of LY038 × MON 810 in the EU is consistently negligible. Therefore, the overall environmental risk posed by this genetically modified higher plant is negligible, and no specific strategies for risk management and no case-specific post-market monitoring actions are considered required.

11.3 Case-specific GM plant monitoring (approach, strategy, method and analysis)

As the overall environmental risk posed by this genetically modified higher plant is negligible, and as the conclusions of the environmental risk assessment are derived from the results of scientific studies, rather than major assumptions, no case-specific post-market monitoring actions, typically aimed at testing assumptions made in this assessment, would be warranted or required.

11.4 General surveillance of the impact of the GM plant (approach, strategy, method and analysis)

Any potential adverse effects of LY038 × MON 810 on human health and the environment, which were not anticipated in the e.r.a., can be addressed under the general surveillance. General surveillance is largely based on routine observation and implies the collection, scientific evaluation and reporting of reliable scientific evidence, in order to be able to identify whether unanticipated, direct or indirect, immediate or delayed adverse effects have been caused by the placing on the market of a genetically modified (GM) crop in its receiving environment.

In order to allow detection of the broadest possible scope of unanticipated adverse effects, general surveillance is performed by either selected, existing networks, or by specific company stewardship programmes, or by a combination of both. Renessen LLC will ensure that appropriate technical information on LY038 × MON 810 and relevant legislation will be available for the relevant networks, in addition to further relevant information from a number of sources, including industry and government websites, official registers and government publications.

Following the approval of this maize, Renessen LLC will approach key stakeholders and key networks of stakeholders of the product (including international grain traders, maize processors and users of maize grain for animal feed) and inform them that import and the use of LY038 × MON 810 as any other maize has been authorized under Regulation (EC) No 1829/2003. Renessen LLC will request key stakeholders and networks for their participation in the general surveillance of the placing on the market of this maize, in accordance with the provisions of Directive 2001/18/EC. Key stakeholders and networks will be requested to be aware of their use of this maize and to inform Renessen LLC in case of potential occurrence of any unanticipated adverse effects to human and livestock health or the environment, which they might attribute to the use of this product. Appropriate technical and safety information on LY038 × MON 810 will be provided to them.

Where there is scientifically valid evidence of a potential adverse effect (whether direct or indirect), linked to the genetic modification, then further evaluation of the consequence of that effect should be science-based and compared with available baseline information. Relevant baseline information will reflect prevalent use practices and the associated impact of these practices on the environment. Where scientific evaluation of the observation confirms the possibility of an unanticipated adverse effect, this would be investigated further to establish a correlation, if present, between the use of LY038 × MON 810 and the observed effect. The evaluation should consider the consequence of the observed effect and remedial action, if necessary, should be proportionate to the significance of the observed effect.

11.5 Reporting the results of monitoring

Renessen LLC will submit an annual General Surveillance Report containing information obtained from participating networks, and/or in case of an effect that was confirmed. If information that confirms and adverse effect which alters the existing risk assessment becomes available, Renessen LLC will submit a Report, consisting of a scientific evaluation of the potential adverse effect and a conclusion on the safety of the product. The report will also include, where appropriate, the measures that were taken to ensure the safety of human or livestock health and/or the environment.

12. Detection and event-specific identification techniques for the GM plant

As LY038 × MON 810 results from traditional breeding of LY038 and MON 810, both inserts are present in combination. Therefore, LY038 × MON 810 is detectable using either the event-specific PCR method for detecting the introduced DNA present in LY038 or the equivalent method for MON 810. However, as for all plants in which two or more inserts are combined by traditional breeding, the unambiguous detection of LY038 × MON 810 in mixed consignments of grain will require single grains to be subjected to the detection methods for both LY038 and MON 810, and to test positive for both.

E. INFORMATION RELATING TO PREVIOUS RELEASES OF THE GM PLANT AND/OR DERIVED PRODUCTS

1. History of previous releases of the GM plant notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

a)	**Notification number** There is no history of release of LY038 × MON 810 in the EU.
b)	**Conclusions of post-release monitoring** Not applicable.
c)	**Results of the release in respect to any risk to human health and the environment (submitted to the Competent Authority according to Article 10 of Directive 2001/18/EC)** Not applicable.

2. History of previous releases of the GM plant carried out outside the Community by the same notifier

a)	**Release country** LY038 x MON 810 has been field tested in the U.S.A. since 2000 and in Argentina since 2001.
b)	**Authority overseeing the release** U.S.A.: United States Department of Agriculture. Argentina: Secretary of Agriculture (SAGPyA) - CONABIA.
c)	**Release site** U.S.A.: mainly in the states of the maize belt, in Hawaii and Puerto Rico. Argentina: Buenos Aires, Cordoba and Santa Fe.
d)	**Aim of the release** U.S.A./Argentina: efficacy, yield, breeding.
e)	**Duration of the release** U.S.A./Argentina: 12 months.
f)	**Aim of post-releases monitoring** U.S.A./Argentina: assess for volunteers.
g)	**Duration of post-releases monitoring** U.S.A./Argentina: 12 months.
h)	**Conclusions of post-release monitoring** U.S.A./Argentina: volunteers have been eliminated to prevent occurrence in subsequent crops.

i)	**Results of the release in respect to any risk to human health and the environment** Field-testing experience provided no evidence that LY038 × MON 810 or derived products would be the cause of any adverse effect to human or animal health, or to the environment.

3. Links (some of these links may be accessible only to the competent authorities of the Member States, to the Commission and to EFSA):

a)	**Status/process of approval** The EFSA website[2] provides information related to the applications submitted under Regulation (EC) No 1829/2003 on genetically modified food and feed.
b)	**Assessment Report of the Competent Authority (Directive 2001/18/EC)** A notification for LY038 × MON 810 according to Directive 2001/18/EC has not been submitted by Renessen LLC.
c)	**EFSA opinion** An EFSA opinion, specifically for LY038 × MON 810, was not available at the time of submission of this application.
d)	**Commission Register (Commission Decision 2004/204/EC)** http://europa.eu.int/comm/food/dyna/gm_register/index_en.cfm
e)	**Molecular Register of the Community Reference Laboratory/Joint Research Centre** Information on the validated detection method for LY038 × MON 810 foods and feeds is posted at http://gmo-crl.it/statusofdoss.htm
f)	**Biosafety Clearing-House (Council Decision 2002/628/EC)** The publicly accessible portal site of the Biosafety Clearing-House (BCH) can be found at http://bch.biodiv.org/
g)	**Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC)** A notification and SNIF according to Directives 2001/18/EC and 2002/812/EC, respectively, have not beet submitted for LY038 × MON 810. The EFSA website[2] does provide a link to this summary of the application for LY038 × MON 810 under Regulation (EC) No 1829/2003.

[2] http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html