Application for authorization to place on the market LY038 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. In countries where LY038 maize[1] will be cultivated, hybrid seed 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.

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 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.

[1] Hereafter referred to as LY038.
™ 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 *ye*s, 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 *n*o, 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 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 application. A summary of the conclusions of the risk analysis that demonstrate the safety of LY038 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 *ye*s, specify	

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

Yes (x)	**No ( )**

If *yes*, specify

LY038 has been notified in the U.S.A. and 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 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 was developed through recombinant DNA techniques by stably integrating into the maize genome the *cordapA* coding sequence under the control of the maize Glb1 promoter to direct the expression of the *Corynebacterium glutamicum*-derived lysine-insensitive dihydrodipicolinate synthase (cDHDPS) protein in the maize germ. The introduction of the *cordapA* gene into maize results in the production of a maize grain with higher lysine content and improved nutritional value for use as a feed ingredient in animal diets, primarily for broiler chickens, turkeys and pigs.

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

This application is for authorization of LY038 for import, food and feed use according to Articles 5 and 17 of Regulation (EC) No 1829/2003 on genetically modified food and feed. It should be noted that LY038 is only intended for use as a feed, but in the unlikely event that LY038 is inadvertently used as a 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 varieties in the EU.

c) Intended use of the product and types of users

LY038 will be a value-added specialty animal feed crop. LY038 will be initially cultivated in Argentina and the U.S.A. It is planned that specific lots of LY038 will be sold per commercial or customer specifications and transported as a specialty crop from the production field, where LY038 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. LY038 is substantially equivalent to traditional maize, except for the introduced lysine trait. This maize was shown to be as safe as traditional maize. Therefore, grain, forage and products produced from LY038 will be stored, packaged, transported, handled and used in the same manner as for current commercial maize, 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 maize is substantially equivalent to traditional maize, except for the introduced lysine trait. Therefore, LY038 and derived products will be used in the same manner as other maize and no specific packaging is required. (For labelling, *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 labelling threshold of 0.9% is applied for the placing on the market of LY038 and derived products.

Operators shall be required to label feeds containing or consisting of LY038 with the words "genetically modified maize" or "contains genetically modified maize", and shall be required to declare the unique identifier REN-ØØØ38-3 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 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 and derived feeds in the EU are required to be aware of the legal obligations regarding traceability and labelling 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 labelling requirements for LY038. 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.

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 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 as any other maize, not including the cultivation of LY038 varieties in the EU, any environmental release would be more likely to occur during import, storage and processing of LY038. However, LY038 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 grain from production to consumption. LY038 grain will be held, transported and handled in a confined manner that will significantly limit the entry of LY038 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 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 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

LY038 was produced by the particle acceleration method using a 5.9 kb linear DNA fragment derived from plasmid vector PV-ZMPQ76, described in detail in Section C.2. This fragment contains the *cordapA* and *nptII* gene cassettes. The *nptII* gene cassette was cloned between two *lox*P sequences to allow for its subsequent removal by the Cre-*lox* recombination system. The 5.9 kb DNA fragment of PV-ZMPQ76 was used in the transformation and was introduced into callus tissue originating from the maize inbred line H99. Maize plants containing the *cordapA* and *nptII* genes regenerated from callus tissue were crossed with plants expressing the Cre recombinase protein. The resulting hybrid underwent excision of the *nptII* gene cassette flanked by the *lox*P sites. The circular *nptII* gene cassette, as well as the Cre recombinase, was subsequently segregated away from plants containing the *cordapA* cassette through additional breeding, which resulted in LY038. Consequently, the DNA inserted in LY038 contains only the *cordapA* gene cassette.

2. Nature and source of the vector used

PV-ZMPQ76 includes three expression cassettes, each with a single copy of a gene: *cordapA*, *nptII* and *AMP*. The *cordapA* gene cassette enables the expression of the dihydrodipicolinate synthase from *Corynebacterium glutamicum* (cDHDPS) predominantly in the grain. The *nptII* gene cassette confers the NptII activity that permits the selection of transformed cells. The third gene cassette contains the *AMP* coding sequence under the control of a bacterial promoter that enables the propagation and selection of the transformed *E. coli* harboring the vector. The genetic elements present in PV-ZMPQ76 are described in more detail in Table 1.

Table 1. Summary of the genetic elements in plasmid vector PV-ZMPQ76

Genetic element	Position in the plasmid	Function and/or reference
Intervening sequence	8773-5	Synthetic linker sequence.
Glb1 promoter	6-1397	The promoter from the *Globulin* 1 (Glb1) gene from *Zea mays*.
Intervening sequence	1398-1404	Synthetic linker sequence.
rAct1 intron	1405-1885	Intron from the rice actin gene.
Intervening sequence	1886	Synthetic linker sequence.
mDHDPS CTP	1887-2057	The chloroplast targeting sequence from dihydrodipicolinate synthase (DHDPS) from *Zea mays*.
cordapA	2058-2960	The coding sequence from the dihydrodipicolinate synthase (*dapA*) gene from *Corynebacterium glutamicum* in the lysine biosynthetic pathway, conferring resistance to lysine feedback inhibition.
Intervening sequence	2961-3036	Synthetic linker sequence.
Glb1 3′ UTR	3037-4036	The 3′ nontranslated region from the *Globulin* 1 (Glb1) gene from *Zea mays* which directs the polyadenylation of the mRNA.

Intervening sequence	4037-4047	Synthetic linker sequence.
lox*P-*2	4048-4081	Recombination site recognized by Cre recombinase.
Intervening sequence	4082-4090	Synthetic linker sequence.
CaMV 35S promoter	4091-4414	Cauliflower mosaic virus (CaMV).
Intervening sequence	4415-4447	Synthetic linker sequence.
nptII	4448-5242	The coding sequence for the enzyme neomycin phosphotransferase type II from Tn*5*, a transposon isolated from *E. coli*.
Intervening sequence	5243-5262	Synthetic linker sequence.
Ble	5263-5415	A 0.153 kb portion of the 0.378 kb *bleomycin* coding sequence from Tn*5*.
Intervening sequence	5416-5426	Synthetic linker sequence.
NOS 3′	5427-5682	3′ nontranslated region of the nopaline synthase (NOS) coding sequence from *Agrobacterium tumefaciens* which directs polyadenylation of the mRNA.
Intervening sequence	5683-5691	Synthetic linker sequence.
lox*P-*1	5692-5725	Recombination site recognized by Cre recombinase.
Backbone sequence	5726-6670	Derived from *E. coli* with polylinker sequences.
AMP	6671-7291	Bacterial promoter and coding sequence for the enzyme ß-lactamase which confers resistance to ampicillin resistance in *E. coli* .
Backbone sequence	7292-8772	Derived from *E. coli* with polylinker sequences.

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

The 5.9 kb DNA fragment used in the maize transformation contains both the *cordapA* and *nptII* gene cassettes. No other plasmid backbone DNA, including the *AMP* gene cassette, was present on this fragment.

In the *cordapA* gene cassette, the cDHDPS coding sequence is under the control of the *Zea mays globulin* 1 (Glb1) promoter, which in wild-type maize directs expression of globulin, the most abundant embryo-specific protein in maize grain. The utilization of the Glb1 promoter for *cordapA* transcription results in directing the expression of the cDHDPS protein and the subsequent accumulation of free lysine to the germ portion of the grain. The intron sequence following the Glb1 promoter is derived from the rice actin 1 gene and the purpose of this element is to enhance DNA transcription. Because cDHDPS is a bacterial enzyme, the *cordapA* coding sequence is preceded by the *Zea mays* dihydrodipicolinate synthase chloroplast transit peptide (mDHDPS CTP), which results in the translation of cDHDPS with the mDHDPS CTP at the N-terminus of the protein. The function of the mDHDPS chloroplast transit peptide is to translocate cDHDPS to the plastid, where lysine biosynthesis occurs via the aspartate pathway. The 3′ nontranslated region of the *globulin* 1 gene following the *cordapA* coding sequence contains the polyadenylation signal that directs the termination and maturation of the *cordapA* transcript.

The *nptII* gene cassette contains the *nptII* coding region regulated by the CaMV 35S promoter and the nopaline synthase 3′ (NOS 3′) transcription termination sequence. As already discussed, the *nptII* gene cassette is not present in LY038.

For additional details on the elements constituting the *cordapA* and *nptII* gene cassettes, please *see* Table 1.

D. INFORMATION RELATING TO THE GM PLANT

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

LY038 contains the *cordapA* gene encoding a dihydrodipicolinate synthase (cDHDPS) from *Corynebacterium glutamicum* conferring increased levels of free lysine in maize grain. Dihydrodipicolinate synthase (DHDPS) 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 into lysine through a series of successive enzymatic reactions. As the first enzyme in lysine biosynthesis, 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.

2. Information on the sequences actually inserted or deleted

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

The data generated by Southern blot analyses support the conclusion that the genome of LY038 contains a single DNA insertion comprised of a single copy of the *cordapA* gene cassette from the 5.9 kb DNA fragment used for the maize transformation.

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

The inheritance of the lysine trait in LY038 follows Mendelian principles. This indicates that the insert is stably integrated in the nuclear genome and is neither located in the mitochondria nor in the chloroplasts.

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

Data generated by Southern blot analyses support the following conclusions:

a) The *cordapA* gene cassette and its associated genetic elements are intact within the single insert;

b) No additional elements from vector PV-ZMPQ76, linked or unlinked to the intact gene cassette, were detected in LY038;

c) LY038 does not contain either intact or partial fragments of the *nptII* gene cassette.

The organization of the elements within the insert in LY038 was confirmed using PCR analysis.

3. Information on the expression of the insert

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

The expression levels of the cDHDPS protein in LY038 tissues were determined by enzyme-linked immunosorbent assay (ELISA).

In 2002, field trials were conducted in the U.S.A. in five sites with different environmental and agronomic conditions representative of the areas where LY038 is expected to be produced commercially. Field production was conducted using agronomic practices typical of maize cultivation. At each site, three replicated plots of LY038 and its control were planted using a randomized block field design. Grain, forage, whole plant, forage root, root and pollen samples were collected once during the growing season. In addition, overseason leaf tissue samples, OSL 1 to 4, were harvested four times during the growing season at V2-V4, V6-V7, V11-V12 and V13-18 plant growth stage, respectively.

The mean cDHDPS protein levels across five sites in LY038 on a dry weight basis in grain, forage, whole plant (V2-V4), forage root, root (V2-V4), and pollen tissues were 26, 0.94, 0.080, 0.069, 1.5, and 0.78 µg/g dw, respectively. Levels of cDHDPS protein in LY038 leaf tissues (OSL 1-4) harvested at four time points throughout the growing season were each less than the assay limit of detection for leaf tissue.

Additional information on cDHDPS expression levels was obtained from field trials conducted in Argentina in the 2001-2002 growing season. Four sites were selected in geographical regions where LY038 would be grown commercially. Each site had three replicated plots. LY038 and its control were planted based on a randomized, complete block field design.

The mean cDHDPS protein levels across four sites for LY038 grain, pollen, forage and forage root tissues were 41, 0.75, 0.19 and 0.12 µg/g dw, respectively. The level of cDHDPS protein in LY038 young leaf tissues leaf tissues was less than the assay limit of quantification.

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 analysis of the cDHDPS expression levels in different maize tissues confirmed that the insert is expressed mainly in LY038 grain.

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 performed with LY038 have demonstrated that LY038 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 agronomic or phenotypic characteristics are expected in LY038 and that LY038 is equivalent to traditional maize in its phenotypic and agronomic behavior.

b) Dissemination

The introduced trait has 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 that cannot survive without the aid of human cultivation practices. Field observations have demonstrated that LY038 has not been altered in its survivability when compared to traditional maize.

d) Other differences

Comparative assessments of phenotypic and agronomic characteristics of LY038 and traditional maize hybrids in the field did not reveal any biologically significant differences.

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

All the observations made, including Chi-square analysis and Southern blot analysis are consistent with Mendelian inheritance of the lysine trait and demonstrate that the DNA insert in LY038 is stably integrated in the nuclear maize genome and consistently expressed over multiple generations.

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 LY038 is unchanged compared to traditional maize and that pollen production and pollen viability were unaffected by the genetic modification, 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, when compared to traditional maize varieties.

However, the scope of the current application does not include the cultivation of LY038 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 was compared to LY038(-), a traditional maize hybrid, whose background genetics is representative of LY038 (*i.e.* the control).

7.2 Field trials

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

LY038 will initially be cultivated in Argentina and in the U.S.A. Therefore, replicated plot, multi-site field trials with LY038, its control and a number of traditional maize hybrids that are commercially important to each of these countries were conducted at four sites in Argentina (three replicates) in the 2001/2002 growing season and at five sites in U.S.A. (three replicates) in the 2002 growing season.

The results of two years of compositional analysis on LY038 maize grown in Argentina and in the U.S.A., at several locations representative of the area where LY038 will be cultivated, concluded that LY038 is compositionally equivalent to traditional maize, except for the intended increase in lysine and the associated increase in two lysine-related catabolites, saccharopine and α-aminoadipic acid, in LY038 grain.

b) the baseline used for consideration of natural variations

In the 2001/2002 Argentinean field trials, four traditionally commercially available maize hybrids were grown at each of the four sites to provide a total of 16 traditional reference hybrids (15 unique reference hybrids).

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). In both cases, 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 and 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

LY038 maize plants expressing the cDHDPS protein under the control of the Glb1 promoter accumulate lysine in the grain. The results of field trials conducted in the U.S.A. show no phenotypic and agronomic differences between LY038 and traditional maize indicating that the LY038 plant phenotype was not altered as a result of the genetic modification.

7.5 Product specification

The *cordapA* coding sequence integrated into the LY038 genome encodes the cDHDPS protein, which results in the accumulation of lysine in LY038 grain. The presence of the lysine trait in maize grain or in maize grain-derived products can be identified by employing different techniques. Southern blot or PCR techniques can identify the inserted nucleotide sequence, while specific ELISAs have been developed to detect the presence of the cDHDPS protein in specific tissues. An event-specific PCR assay allowing the detection and the quantification of LY038 has been developed and is provided to the Joint Research Center (JRC), acting as the Community Reference Laboratory (CRL).

7.6 Effect of the production and processing

Due to its nutritional characteristics, LY038 is destined exclusively for animal feed uses. The effects of production and processing of LY038 are not expected to be any different from the production and processing of the equivalent feed materials originating from traditional maize, since LY038 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 the unlikely case that adventitious, trace amounts of LY038 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 is intended to be used as a value-added specialty animal feed crop. LY038 will be initially cultivated in Argentina and the U.S.A. It is planned that specific lots of LY038 will be sold per commercial or customer specifications and transported as a specialty crop from the production field, where LY038 is grown, to specific end customers (feed manufactures and livestock producers) in the EU. LY038 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.

7.8 Toxicology

7.8.1 Safety evaluation of newly expressed proteins

The safety assessment of the cDHDPS protein expressed in LY038 leads to the following conclusions:

a) The donor organism, *Corynebacterium glutamicum*, is a common soil bacterium widely distributed in the environment and is not an animal or human pathogen;

b) A history of safe use of the cDHDPS protein has been demonstrated, based on the similarity of the cDHDPS protein in LY038 to DHDPS proteins naturally present in feed and food (e.g., maize, rice, soy and wheat);

c) No biologically relevant structural similarities were observed between the cDHDPS protein and toxins, allergens or pharmacologically active proteins that are known to cause adverse health effects in animals or humans;

d) The cDHDPS protein is rapidly degraded in simulated gastric fluid, indicating that it is unlikely to elicit allergenic or toxic effects;

e) The cDHDPS protein purified from *E. coli* was found to be physicochemically and functionally equivalent to the cDHDPS protein produced in LY038;

f) The results of a mouse acute oral toxicity study demonstrate that the cDHDPS protein is not toxic at the highest dose tested of 800 mg/kg BW/day and does not cause any observable effects;

g) Margins of safety calculation indicate that there is unlikely to be any meaningful risk to animal and human health resulting from the dietary intake of the cDHDPS protein.

7.8.2 Testing of new constituents other than proteins

LY038 has been genetically modified by inserting in the maize genome the *cordapA* gene expressing the cDHDPS protein. No other new constituent, apart for the cDHDPS protein, has been targeted for expression in this product. 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 was shown to be substantially equivalent to traditional maize (except for the introduced lysine trait), no testing of any constituent other than the introduced protein is indicated.

7.8.3 Information on natural food and feed constituents

LY038 grain and forage are compositionally equivalent to traditional maize grain and forage, with the exception of the intende increase in lysine and the associated increase in two lysine-related catabolites, saccharopine and α-aminoadipic acid, in LY038 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 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 grain. The safety of the cDHDPS protein, lysine, saccharopine and α-aminoadipic acid for humans and animals has been demonstrated. The safety of LY038 has been further confirmed by a 90-day feeding study in rats conducted with LY038 whole-grain maize and by a 42-day feeding study in broilers fed LY038 grain.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

The allergenicity potential of the cDHDPS protein was assessed by bioinformatic comparison of the amino acid sequence of cDHDPS with a database of known allergen sequences, as well as evaluation of the stability of the protein in an *in vitro* gastric digestion model.

The bioinformatic analyses of the cDHDPS protein sequence revealed no biologically relevant structural or immunological similarities to known allergens. Furthermore, no short (eight amino acid) polypeptide matches were shared between the cDHDPS protein sequence and proteins in the allergen database. These data indicate the lack of both structurally and immunologically relevant similarities between allergens and the cDHDPS protein sequence. The demonstrated rapid degradation of the cDHDPS protein in simulated gastric fluid provides further evidence of the lack of allergenic potential of the expressed cDHDPS protein.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

As the introduced protein does not have allergenic potential, it was concluded that the use of LY038 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 7.10.2

7.10.2 Nutritional assessment of GM feed

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

The nutritional value of LY038 was assessed by a 42-day broiler feeding study. The bioefficacy and bioavailability of the incremental lysine expressed in LY038 grain was demonstrated by performance and carcass measurements of birds receiving a diet formulated with LY038, 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, or traditional reference maize included at the same inclusion rate as LY038).

Enhanced growth, feed efficiency and carcass yield due to the increased level of available lysine in LY038 grain were demonstrated by the observed superior performance of broilers fed a diet containing LY038 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 grain. Therefore, LY038 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.

7.11 Post-market monitoring of GM food/feed

There are no intrinsic hazards related to LY038 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 demonstrates that the risks of consumption of LY038 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.

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 spreading in the environment is negligible, as maize is neither persistent nor invasive and LY038 is equivalent to traditional maize in its phenotypic and agronomic behavior. In the unlikely event of establishment of a LY038 plant, *e.g.* from a grain spilt in the environment, its introduced trait would have negligible consequences for the environment. Hence the risk of unintentional spreading of LY038 through increased weediness of this maize is negligible.

9.2 Selective advantage or disadvantage

Compared to traditional maize, any newly introduced characteristics in LY038 are limited to the lysine trait. It was demonstrated previously that the introduced genetic sequences in LY038 did not lead to any biologically meaningful alterations of other phenotypic characteristics, such as plant growth and development, morphology and agronomic performance, when compared to traditional maize. Therefore, the assessment of any competitive (dis)advantages of LY038 will be limited to the lysine trait imparted by the expression of the cDHDPS protein, which results in the intended increase in lysine and in the associated increase of the two lysine-related catabolites, saccharopine and α-aminoadipic acid, in LY038 grain. The lack of toxicity of the cDHDPS protein to human and animals has been extensively demonstrated.

Therefore, the presence of this protein in the maize plant is not expected to confer a meaningful selective advantage or disadvantage in the interaction of LY038 with the environment. Lysine is an essential amino acid in proteins. Saccharopine and α-aminoadipic acid are natural catabolites of lysine metabolism in humans, animals and plants and are present in commonly used feeds and foods. The increase in lysine and in the two lysine-related catabolites, saccharopine and α-aminoadipic acid, is not anticipated to offer this maize any selective advantage or disadvantage.

Even when spillage of LY038 grains would result in the short survival of some maize volunteers, it would not represent a meaningful advantage over wild plants since LY038, like any other maize, is a poor competitor in European conditions and the likelihood for spilt maize kernels to survive and establish is negligible. Also, in the case of germination of any spilt grain (which will be of the F2 generation) or in the unlikely case of misuse of the grain for planting, the fitness of the resulting plants would be expected to be less than typical, commercially available maize hybrid cultivars. Reduced fitness would result from the fact that, as with all F1 hybrid cultivar maize seed, F1 hybrid cultivars of LY038 do not "breed true". Consequently, the growth, development and yield of the resulting (F2) plants is variable and predominantly reduced in vigor, their morphology often resembling the less vigorous inbred lines from which the F1 seed was produced. Furthermore, such plants could readily be controlled by mechanical means or by one of a number of the other graminicides in current use.

9.3 Potential for gene transfer

There is no potential for gene transfer from LY038 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 in the EU. In the highly unlikely event that the *cordapA* gene would outcross to another maize plant, the environmental risk posed by this gene transfer is negligible.

9.4 Interactions between the GM plant and target organisms

LY038 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 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 trait present a negligible hazard to non-target organisms, even if incidental spillage of LY038 grains during import, storage, transport or use would lead to the short survival of LY038 plants in the environment. As a consequence, there is negligible risk for harmful effects of LY038 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 contains the cDHDPS protein, which has 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 and in humans, consuming those animals, is negligible. Therefore, the risk of LY038 for the food/feed chain is also negligible.

9.8 Effects on biogeochemical processes

In the event of an incidental release of LY038 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 plants would be any different from traditional maize regarding their direct influence on biogeochemical processes or nutrient levels in the soil, as LY038 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, 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 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 was shown to be substantially equivalent to traditional maize, with the exception of the intended increase in lysine and in the associated increase in the two lysine-related catabolites, saccharopine and α-aminoadipic acid, conferred by the expression of the introduced cDHDPS protein.

The cDHDPS protein is innocuous, belonging to a class of proteins that are ubiquitous in nature, and has a history of safe use with no known negative interaction with the environment. Lysine is an essential amino acid in proteins. Saccharopine and α-aminoadipic acid are natural catabolites of lysine metabolism in humans and animals and are present in commonly used foods and feeds.

No deleterious impact of LY038 on the abiotic environment is expected to result from the import, processing or use of this product for feed in the EU.

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.) was conducted for LY038 according to the principles laid down in Annex II to Directive 2001/18/EC. The e.r.a. was undertaken in the context of the scope of this application under Regulation (EC) No 1829/2003, that is, for import, food and feed use of LY038 in the EU, but excluding the cultivation of LY038 varieties in the EU. Analysis of the characteristics of LY038 has shown that the risk for potential adverse effects on human or animal health and on the receiving environment, resulting from the import and use of LY038 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 this 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 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 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 in the EU, 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 the product has been authorized. Renessen LLC will request key stakeholders and networks for their participation in the general surveillance of the placing on the market of this maize. 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 health or the environment, which they might attribute to the import or use of this product. Appropriate technical information on LY038 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 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 a General Surveillance Report containing information obtained from participating networks, and/or in case of an effect that was confirmed. If information that confirms an 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

The LY038 insert is detectable in food and feed using the validated, event-specific PCR method for detecting the introduced DNA present in LY038, provided to the Joint Research Center (JRC), acting as the Community Reference Laboratory (CRL).

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 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 has been field tested in the U.S.A. since 2000, in Argentina since 2001, in Japan in 2003, in Brazil in the 2003-04 growing season and in China in 2002.
b)	**Authority overseeing the release** U.S.A.: United States Department of Agriculture. Argentina: Secretary of Agriculture (SAGPyA) - CONABIA. Japan: Ministry of Agriculture Fisheries and Forestry. Brazil: National Technical Commission on Biosafety (CTNBio). China: Ministry of Agriculture.
c)	**Release site** U.S.A.: mainly in the states of the maize belt and in Hawaii and Puerto Rico. Japan: Kawasaki prefecture. Argentina: Buenos Aires, Cordoba, Santa Fe. Brazil: Santa Cruz das Palmeiras, SP; Santa Helena de Goias, GO; Rolandia, PR; Ponta Grossa, PR. China: Beijing, Hebei, Jilin, Liaoning, Henan and Shandong.
d)	**Aim of the release** U.S.A./Argentina/Brazil/China: efficacy, yield, breeding. Japan: stage III environmental assessment.

e)	**Duration of the release** U.S.A./Argentina/Brazil/China/Japan: 12 months.
f)	**Aim of post-releases monitoring** U.S.A./Argentina/Brazil: assess for volunteers.
g)	**Duration of post-releases monitoring** U.S.A./Argentina: 12 months. Brazil: 6 months.
h)	**Conclusions of post-release monitoring** U.S.A./Argentina/Brazil: 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 provided no evidence that LY038 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 according to Directive 2001/18/EC has not been submitted by Renessen LLC.
c)	**EFSA opinion** An EFSA opinion, specifically for LY038, 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

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

e)	**Molecular Register of the Community Reference Laboratory/Joint Research Centre** Information on detection protocols can be found 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 been submitted for LY038. The EFSA website does provide a link to this summary of the application for LY038 under Regulation (EC) No 1829/2003.