SUMMARY OF APPLICATIONS FOR GENETICALLY MODIFIED MICROORGANISMS AND/OR DERIVED PRODUCTS INTENDED FOR FOOD AND FEED USE

A. GENERAL INFORMATION

1. Details of application

a) Member State of application Not applicable. Renewal of authorization according to Article 23 of Regulation (EC) No 1829/2003 for an 'existing product' according to its Article 20 (1)(a) and (4). Application made to the European Commission.
b) Application number Not yet allocated to the Applicant at the time of the remittance of the dossier to European Commission.
c) Name of the product (commercial and other names) - for the purpose of this dossier: PL73 *Brevibacterium* - commercial name: PL73
d) Date of acknowledgement of valid application Yet to be determined As provided by Article 20(4) of Regulation (EC) No 1829/2003 for products authorised according to its Article 20(1)(a), this dossier applies for a renewal of the authorization of 'PL73 *Brevibacterium'* in accordance with Article 23 of the said Regulation.

2. Applicant

a) Name of applicant AJINOMOTO EUROLYSINE S.A.S., contact person: Philippe GUION
b) Address of applicant 153, rue de Courcelles 75817 PARIS Cedex 17 France
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)) The person established in the Community responsible for the placing on the market will be the Applicant.

3. Scope of the application

☐ GM microorganisms and/or derived products for food use

■ GM microorganisms and/or derived products for feed use

☐ GM microorganisms and/or derived product(s) belonging to Group 1, as defined in Chapter II, 2. of this Guidance

■ GM microorganisms and/or derived product(s) belonging to Group 2, as defined in Chapter II, 2. of this guidance

☐ GM microorganisms and/or derived product(s) belonging to Group 3, as defined in Chapter II, 2. of this guidance

☐ Import and processing (Part C of Directive 2001/18/EC)

4. Is the product being simultaneously notified within the framework of another regulation?

Yes ☐	No ■
A notification was made for this product as exiting product according to article 20(1)(a) of Regulation (EC) No 1829/2003 on 18 October 2004.	

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

Yes ☐	No ■
If *no*, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC	

6. Has the GM microorganism 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 ■
If *yes*, specify	

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

Yes ☐	No ■
If *yes*, specify	

8. General description of the product

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

The product PL73 *Brevibacterium,* subject of the present application, consists of the dried killed cells of a genetically modified strain of *B. lactofermentum/C. glutamicum,* named SO317/pCABL used to produce the amino acid L-lysine (nutritional feed additive).

The strain SO317/pCABL consists of the recipient strain SO317 and one plasmid pCABL. The purpose of this genetic modification was to increase the ability of strain SO317 to produce L-lysine by fermentation on substrates of agricultural origin.

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

The dried killed bacterial biomass PL73 *Brevibacterium* mentioned in a), is a by-product of the production process of L-lysine using strain (SO317/pCABL) and is placed/is intended to be placed on the market as feed material.

c) Intended use of the product and types of users

Among several possibilities to dispose of this by-product, considering its high nitrogen content, and the non-negligible tonnage it represents (10 000 to 15 000 tons per year) its usage as feed material source of crude protein appears the most relevant The use of this feed material, as a source of crude protein, is in compound feedingstuffs formulated for ruminants (including protein concentrates), pigs and fish (salmonids).

The product is sold in pellet form and is delivered in 'bulk' to feed mills only.

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

Handling
Instructions for handling are standard precautions for powdered products or products generating fine dust mentioned in the material safety data sheet. The product may cause sensitisation by inhalation and skin contact (as any protein-containing product).

Storage
The product shall be stored at dry conditions in standard silos and kept away during handling from ignition and heat sources

Use in compound feedingstuffs:
Ruminants from the beginning of rumination
Maximum incorporation level: 100g DM per kg ration DM (or 11-12% product as such per kg feed)

Pig
Maximum incorporation level: 10% ('product as is')

Fish (salmonids)
Maximum incorporation level: 10% ('product as is')

e) Any proposed packaging requirements

Except granulating/pelleting the product will be sold as such, in bulk, to feed mills.

f) A proposal for labeling in accordance with Articles 13 and Articles 25 of Regulation (EC) 1829/2003. In the case of GMOs, food and/or feed containing or consisting of GMOs, a proposal for labeling has to be included complying with the requirements of Article 4, B(6) of Regulation (EC) 1830/2003 and Annex IV of Directive 2001/18/EC

a) As feed material

- Feed material
- *(Name)*: Bacterial protein, by-product from the production of L-lysine by fermentation on agricultural substrate, produced from genetically modified micro-organisms
- Moisture
- Nitrogen expressed as crude protein
- Approval number (Regulation 183/2005)
- All animal species (Annex to French 'Arrêté' of 27 August 1987, column 6)
- Net weight
- Batch number
- Date of production
- Date of expiry

b) Declarations to be made on the label or packaging of compound feeding stuffs

- The name: 'Bacterial protein, by-product from the production of L-lysine by fermentation on agricultural substrate, produced from genetically modified micro-organisms'
- Percentage of the total crude protein provided by non-protein nitrogen

As the product is delivered in bulk to feed mills (delivery by means of tank trucks), the information corresponding to labelling is provided to customers by means of the commercial documents preceding or accompanying the delivery of the product (taking into account the official language of the country of destination) and the commercial technical sheet corresponding to this product.

g) Unique identifier for the GM microorganism in accordance with Regulation (EC) 65/2004

Not applicable.
Strain SO317/pCABL is used for the production of L-lysine in containment only.
The manufacturing process of L-lysine and of the by-product / dried killed bacterial biomass 'PL73 *Brevibacterium'* ensures that the final product does not contain viable cells nor transferable DNA of the L-lysine producer GM microorganism.

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

The renewal of authorization sought by the Applicant for the use as feed material of the product PL73 *Brevibacterium* concerns the EU market.

Environment to which the product is unsuited: not applicable.

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

The product PL73 *Brevibacterium* does not contain viable cells, nor transferable DNA of the L-lysine-producer GM microorganism. Therefore, it is not necessary to take measures, because no unintended release or misuse is expectable.

B. INFORMATION RELATING TO THE GMM

1. Characteristics of the recipient or (when appropriate) parental organism

1.1 Identity

a) Common name Not really applicable. The recipient strain belongs to the species *Brevibacterium lactofermentum/Corynebacterium glutamicum*.Its denomination 'SO317' is internal to the Ajinomoto Group.
b) Strain designation Strain SO317 is only a laboratory strain used for the construction of the final L-lysine producer microorganism. Its designation is internal to the Ajinomoto Group.
c) Source of the strain Strain SO317 derives from the parental *Brevibacterium lactofermentum/Corynebacterium glutamicum* strain 2256 through several steps of genetic modification.
d) Accession number from a recognized culture collection Strain SO317 is part of Ajinomoto Group collection and has not been deposited The original parental strain, named 2256, has been deposited under the following numbers: - ATCC N°13869, - NCIMB N° 9567

1.2 Taxonomy

a) Genus Corynebacterium
b) Species Corynebacterium glutamicum
c) Subspecies

Not applicable

d) Strain

- Initial parental strain (see B.1.1d): 2256
- recipient strain: SO317

A genetic analysis was performed on the initial 2256 strain and on the lysine producer SO317, using a reference strain of the 'Institut Pasteur', to confirm the descent from *B. lactofermentum.*
A ribotyping analysis was also performed by 'Institut Pasteur' on the parental strain 2256 and on the lysine producer (SO317) which showed identical (or almost identical) patterns for DNA of strains 2256 and SO317 with each of four restriction endonucleases used (see also B.1.14)

1.3 Other names

There are no other names for the recipient strain. Although the name *C. glutamicum* should now be used for the species to which strains 2256 and SO317 are belonging, in the dossier the 'historical' name *B. lactofermentum* is used.

1.4 Phenotypic and genetic markers

a) Phenotypic and genotypic information relevant to identification, genetic stability and safety

B. lactofermentum lysine producer:
- Single cells, which are Gram-positive and not sporulating
- Colony: size around 1 to 3 mm, rounded, smooth area an white-cream colour
- facultative anaerobic microorganism
- auxotrophic for two vitamins: biotine and thiamine

b) Information on pathogenicity

Confirmation of the absence of pathogenicity (infectivity, toxigenicity, virulence), has not been made on the recipient strain SO317 per se, but on *B. lactofermentum/C. glutamicum* species through a literature search over more than 30 years. (see B.1.11d).
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1.5 Degree of relatedness between recipient and donor(s), when appropriate

This information is considered as confidential by the Applicant and is, therefore, not included in this summary.

1.6 Description of identification and detection techniques

Ribotyping is used as detection technique (see B.1.2).

1.7 Sensitivity, reliability and specificity of the detection techniques

This section is nor relevant for the parental strain *B. lactofermentum*, nor the recipient strain SO317 because they are used in laboratory only.

1.8 Source and natural habitat of the recipient microorganism

This section is not applicable to final products deriving from GMM falling within 'Group 2' according to the EFSA Guidance document (see also C.2.2 and C.4).
Nevertheless it may be indicated that corynebacteria (including *B. lactofermentum*) used for amino acids production were originally isolated from the soil.

1.9 Organisms with which transfer of genetic material is known to occur under natural conditions

An endogenous and cryptic plasmid was described in *B. lactofermentum* strain 2256. Additional information concerning the recipient strain SO317 is considered as confidential by the Applicant and is, therefore, not included in this summary.

1.10 Information on the genetic stability of the recipient microorganism

There is no evidence of instability of the recipient strain (see also section B.5.3).

1.11 Pathogenicity, ecological and physiological traits

a) Classification of hazard according to the current Community legislation

French Expert Committee CGG has classified strains L-lysine producing strain SO317/pCABL as Group I, Class I strain for the contained use/production, which means it does not present any hazard to human and environment (CGG opinion of July 1996, dossier registered under the number N°E-96-06-02).

b) Information on the doubling time and of the mode of reproduction

B. lactoferementum/C. glutamicum has a doubling time of less than one hour. However, it is strongly increased in the case of amino acid-producing strain (several hours). The mode of reproduction is the vegetative form.

c) Information on survival, ability to form spores or other survival structures

B. lactofermentum is a non-spore forming microorganism

d) Infectivity

A literature search over more than 30 years was performed using Boolean operators "(glutamicum OR lactofermentum) AND infection" and the same with "isolation" instead of "infection" was performed by Pasteur Institute (Paris). This search yielded no paper dealing with the isolation of the organism in humans and none dealing with animal infection.

e) Presence of genes that confer antibiotic resistance

See pCABL plasmid construction in Part B.3.1.

f) Involvement in environmental processes

Not applicable, because the recipient strain SO317 is only an intermediate strain, used at laboratory level, for the construction of the Lysine-producing strain SO317/pCABL.

1.12 Information on indigenous mobile genetic elements

This information concerning the recipient strain SO317 is considered as confidential by the Applicant and is, therefore, not included in this summary.

1.13 Description of its history of use

B. lactofermentum has been re-classified as *C. glutamicum* or *C. lactofermentum*. This microorganism has been used for over 30 years in the production of food additives and amino acids for human (medical) nutrition and animal nutrition. Glutamic acid and flavour enhancing nucleotides are typical fermentation products obtained using this microorganism. Some pharmaceutical amino acids, are or have also been produced using this microorganism. Based on this long historical usage without any known adverse effects, this microorganism could be recognised, using the US approach, as a non-pathogenic GRAS microorganism.

1.14 History of previous genetic modifications

Strain SO317 derives from the initial parental *B. lactofermentum* strain through several steps of genetic modification. This information is considered as confidential by the Applicant and is, therefore, not included in this summary.

2. Characteristics of the donor organism(s)

All the information concerning this section B.2 is considered as confidential by the Applicant and is, therefore, not included in this summary.

2.1 Identity

a) Common name
b) Strain designation
c) Source of the strain
d) Accession number from a recognized culture collection

2.2 Taxonomy

a) Genus
b) Species
c) Subspecies
d) Strain

2.3 Other names

a) Generic name
b) Commercial name
c) Previous name(s)

2.4 Phenotypic and genetic markers

a) Phenotypic and genotypic information relevant to identification, genetic stability and safety
b) Information on pathogenicity

2.5 Description of identification and detection techniques

2.6 Sensitivity, reliability and specificity of the detection techniques

2.7 Source and habitat of the organism

2.8 Pathogenicity traits

a) Classification of hazard according to the current Community legislation
b) Pathogenicity
c) Infectivity
d) Toxigenicity
e) Virulence
f) Allergenicity

g) Ability to act as carrier of pathogenicity islands

2.9 Description of its history of use

3. Description of the genetic modification process

3.1 Characteristics of the vector

The strain SO317 has been transformed by the plasmid pCABL. Vector sequences of the plasmid are widely used in recombinant DNA experiments. Each fragment inserted is well characterised and sequenced. Detailed information on plasmid pCABL and all the information on the structure of the plasmid and on the vector effects are considered as confidential information and is, therefore, not included in this summary.

3.2 Information relating to the genetic modification

The information relating to the genetic modifications of the recipient strain and on the function of the main sequences inserted in the plasmid pCABL are considered as confidential information and is, therefore, not included in this summary.

4. Identification of the conventional counterpart microorganism and its characteristics

Not applicable for GM product falling within Group 2 according to the EFSA guidance document for this application.

5. Information relating to the GMM and comparison of the GMM with its conventional counterpart

5.1 Description of the genetic trait(s) or phenotypic characteristics and any new trait which can be expressed or no longer expressed

The morphology and the biological properties of the lysine producing strain obtained (SO317/pCABL) are the same as those described for the recipient strain SO317, which is provided in section B.1 of this summary. There is additional genotypic and biological property information on the final strain SO317/pCABL, but this is considered as confidential information and is, therefore, not included in this summary.

5.2 Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified microorganism

This information is considered as confidential information and is, therefore, not included in this summary.

5.3 Stability of the microorganism in terms of genetic traits

The stability of the last genetic modification (insertion of plasmid pCABL in recipient strain SO317) is analyzed by different approaches:

- Lysine production ability by fermentation tests (without any selective pressure and number of generations higher than 15).
- Measurement of the percentage of colony forming units containing the plasmid on selective medium on cell suspensions after fermentation tests. For the plasmid, the percentage of colony forming units containing the plasmids is close to 100%.
- Analysis, after digestion by restriction enzymes and electrophoresis, of the plasmid extracted from cell suspensions after fermentation tests. The analysis of plasmid does not show any visible anomaly which could result from deletions, or intra or inter plasmidic recombinations.

This reflects the good segregational and structural stability of the plasmid, sine qua non conditions for a high lysine production.

5.4 Rate and level of expression of the new genetic material

Not applicable to final products deriving from GMM falling within Group 2 according to the EFSA guidance document for this application.

5.5 Description of identification and detection techniques

The method for detection and identification proposed for the dried bacterial biomass 'PL73 *Brevibacterium'* could be applied to strain SO317/pCABL for its identification.

5.6 Information on the ability to transfer genetic material to other organisms

The most important point for the evaluation of the ability to transfer genetic material to other organisms is the presence of extrachromosomal replicon containing the kanamycin resitance gene. In fact, except the *npt*I gene coding for kanamycine resistance, the other genes present in the plasmid are genes related to amino acid metabolism. However, this plasmid was constructed from non-conjugative plasmid and, as indicated in section B.3, the plasmid does not present homologous sequences with mod and tra sequences.

Moreover, an adequate inactivation process was developed and is applied to the 'broth out' at the completion of the fermentation phase to ensure the absence of viable cells and transferable DNA of the L-lysine producing microorganism in the final product PL73 *Brevibacterium*. As described in section C.4, the DNA of lysine-producing strain SO317/pCABL is degraded extensively by the 'inactivation process', and this results in the presence of non functional DNA in the final product PL73 *Brevibacterium*.

5.7 Information on the interaction of the GMM with other organisms

Not applicable to final products deriving from GMM falling within Group 2 according to the EFSA guidance document for this application.

5.8 History of previous releases or uses of the GMM

Not applicable to the GMM used to produce the dried killed bacterial biomass PL73 *Brevibacterium*. This microorganism has not been the subject of previous releases / uses as live microorganisms in non-contained conditions

5.9 Safety for humans and animals

a) Information on any toxic, allergenic or other harmful effects on human or animal health

It may be stated that, considering the nature of the modifications made to obtain the strain SO317/pCABL, the characteristics of the L-lysine / PL73 *Brevibacterium* manufacturing process, no toxic, allergenic or other harmful effects on human or animal health are to be expected from the L-lysine producing microorganism.

As regards the resistance to kanamycin of the strain resulting from its construction, DNA is degraded by the 'inactivation process' which results in the presence of non functional DNA in the final product PL73 *Brevibacterium* (see section C.4).

b) Potential for DNA transfer or any capacity for enhanced gene transfer

This aspect is addressed in section C.4 of this summary.

c) Viability and residence time of the GMM in the alimentary tract

Not applicable to PL73 *Brevibacterium* deriving from the GMM as this biomass does not contain viable cells of the GMM (see sections C.2.2 and C.4).

d) Information on any impact of the GMM on the microbiota of the human or animal gastrointestinal tract

Not applicable to PL73 *Brevibacterium* deriving from the GMM as this biomass does not contain viable cells of the GMM (see sections C.2.2 and C.4).

5.10 Information on monitoring, control, waste treatment and emergency response plans

This section is not applicable to final products deriving from GMM falling within Group 2 according to the EFSA guidance document for this application.

C. INFORMATION RELATING TO THE GM PRODUCT

1. Information relating to the production process

PL73 *Brevibacterium* is a by-product of the manufacturing process of L-lysine/ L-lysine-based nutritional feed additives by fermentation on agricultural substrates. L-lysine is produced by fermentation process ('fed-batch fermentation') of a selected strain of *B. lactofermentum* strain which has been modified to produce L-lysine.
The fermentation culture medium consists of carbon sources (sugars), nitrogen sources, inorganic salts, amino acids and vitamins. Other substances which are present during manufacturing are acids, bases, resins and an antifoaming agent (added during fermentation).
The production of PL73 *Brevibacterium* consists of the following steps (chronological): strain preservation, culturing of seeds, and fermentation using the sterilised raw materials of the fermentation culture medium. Afterwards the broth is inactivated and subjected to further processing containing the following steps (chronological): recovery and washing of the inactivated cells of the L-lysine producer, decantation and concentration of the bacterial cells, cell drying, granulation, cooling, sieving and storage. The constancy and purity of the lysine-producing strain are strictly monitored.

2. Information relating to the product purification process

2.1 Technique used to remove microbial cells from the product

Not applicable since the microbial cells are not removed from the product. PL73 *Brevibacterium*, in essence, contains inactivated and denaturated microbial cells.

2.2 Information on the technique used to kill the microbial cells

Lysine broth and washing liquor of the fermenter are received from the fermenter through a broth pipeline into a 'broth tank' by air blow. This broth is then submitted to a physico-chemical treatment to inactivate and denature the cells of the lysine-producer microorganism (SO317/pCABL) it contains.

Verification of the absence of viable cells
After inactivation, a bacteriological analysis is carried out on the inactivated broth to look for the potential presence of viable recombinant cells. No viable cells of the lysine producer organism are detected during this analysis.

2.3 Information on the process used to purify the product from the microbial growth medium

This section, mainly introduced for 'purified' products, such as amino acids, enzymes, etc., is not considered applicable to products such as PL73 *Brevibacterium*.
It may be indicated that the inactivated bacterial cells making up PL73 *Brevibacterium* are washed during their recovery.

3. Description of the product

3.1 Designation of the product

PL73 *Brevibacterium* is a dried killed bacterial biomass, which is a by-product of L-lysine production by fermentation using a genetically modified strain of *B. lactofermentum* and is used as feed material.
PL73 *Brevibacterium* has a complex nature and does not contain viable cells nor transferable DNA of the GMM. The current commercial name of PL73 *Brevibacterium* is PL73.

As PL73 *Brevibacterium* is delivered in bulk to feed mills, the information corresponding to labelling is provided to customers by means of the commercial documents preceding or accompanying the delivery of PL73 *Brevibacterium* (taking into account the official language of the country of destination) and the commercial technical sheet corresponding to this product.

3.2 Intended use and mode of action

The main outlet of PL73 *Brevibacterium* is ruminant nutrition (as a direct or indirect source of protein), from the beginning of rumination. Its maximum level of incorporation in feedingstuffs is: 100g DM per kg ration DM (or 11-12 % product as such per kg feed). In practice, the level of use in feeds is usually in the range of 5 to 8% (product as such), but may be occasionally higher.
Other outlets, to a lesser extent, are compound feedingstuffs for pig (grower – finisher) and fish feeds (salmonids).

3.3 Composition

A compositional analysis was performed for the following parameters to determine its main and minor components (also in view of assessing its nutritive value) and the occurrence of potential contaminants:

* Crude components
* Nitrogen components (total and free amino acids, ammonium N, amide N, urea N, biogenic amines, nitrates and nitrites, nucleic acids)
* Total lipids, fatty acids
* Carbohydrate fraction
* Organic acids
* Inorganic components
* Vitamins
* Potential contaminants: heavy metals, organochlorine and organophosphorus pesticides, dioxins, PCBs, polyaromatic hydrocarbons

Residues of solvents were not analysed as no solvent is used in the lysine /PL73 *Brevibacterium* manufacturing process.
Quality control analyses are performed on an average sample on the above mentioned parameters every month, except for dry matter (humidity), total nitrogen (and crude protein = N total x 6.25) and ammonium N, which are analysed daily.

3.4 Physical properties

PL73 *Brevibacterium* is a slightly brown to brown product with a pH of 5.0 (in 10 % w/v suspension).

- Electrostatic properties: MIE > 1200 mJ
- Auto-ignition: 500 ^{o}C
- Explosivity:
  Pmax: 6.2 bar
  MRPmax: 370 bar/s
  Kst: 100 bar
  Explosion class: St1

3.5 Technological properties

The effects of different climatic conditions - combinations of different temperatures and relative humidity (RH) - on the behaviour and stability of PL73 *Brevibacterium* were investigated:
- PL73 *Brevibacterium* was chemically stable during 12 months storage at 5 different climatic conditions, covering a wide range of moderate and subtropical climate conditions. PL73 *Brevibacterium* demonstrated a good microbiological quality during the storage period and did not contain pathogenic micro-organims. Therefore, it can be considered as microbiologically safe feed material.
- Dairy concentrates containing max. 20% PL73 *Brevibacterium* were chemically stable during 6 months storage at 3 different climatic conditions, covering a realistic range of moderate and subtropical climatic conditions. Furthermore, they did not contain pathogenic microorganisms at hazardous levels.

4. Assessment of the presence of recombinant DNA and of the potential risk of gene transfer

Research of living cells of strain SO317/pCABL
PL73 *Brevibacterium* contains recombinant DNA coming from the strain SO317/pCABL (detected by PCR). The quantity of target molecules present in the form of degraded plasmidic DNA is equivalent to that which would be present in 1pg of plasmidic DNA for 0.01 mg of product (or 50pg of plasmidic DNA for 0.5 mg of product). However, this DNA is in a degraded form. The size of the DNA fragments (< 2.2 kb) is significantly smaller than the size of the pCABL plasmid.

The presence of transformant DNA was not detected in PL73 *Brevibacterum* in using a transformation system in both *E. coli* and *B. lactofermentum*. No kanamycin resistant transformant was obtained in using up to 30 mg and 22 mg of PL73 *Brevibacterium* extracted according to two protocols.

The status of PL73 *Brevibacterium* with regard to Directive 90/220/EEC (meanwhile replaced by Directive 2001/18/EC) was examined by the French Commission du Génie Biomoléculaire (CGB) in 1997. In its opinion, CGB considered that 'in the production conditions implemented, the by-product of the production of L-lysine described in the dossier is not a GMO within the meaning of Law n° 92-654 of 13 July 1992 on the control of the use and of the dissemination of genetically modified organisms'.

Risk assessment of the marker gene used in the recombinant amino acid producer strain
Adverse effects on the therapeutic use of antibiotics corresponding to the marker gene used in the strains are not envisaged upon the use of the feed materials (amino acids and amino acid by-products). This conclusion

is based on the following facts: viability of the strain reduced during production, DNA integrity is strongly affected by the depurinating effect of pH values below 4.5 and/ or heat treatment, which abolish the transformation of potential plasmids and the marker gene is widely distributed in nature.

5. Comparison of the GM product with its conventional counterpart

This section is not applicable to the dried killed bacterial biomass PL73 *Brevibacterium*, placed on the market as feed material, because no biomass resulting from the production of lysine using a conventional strain of *Brevibacterium* is anymore manufactured for nearly a decade and placed on the market. A comparative risk assessment with a conventional counterpart is therefore not possible. A full risk assessment has therefore been carried out.

6. Considerations for human health and animal health of the GM product

6.1 Toxicology

The safety of PL73 *Brevibacterium* was evaluated with several studies. Here the main conclusions of these studies are presented.

Laboratory animals:

* Acute toxicity studies

- The acute oral toxicity of PL73 *Brevibacterium* in rats has an LD_{50} > 2000 mg/kg body weight. PL73 *Brevibacterium* is not harmful when ingested.
- The acute inhalation toxicity of PL73 *Brevibacterium* in rats has an LD_{50} > 5.22 g/m^3. PL73 *Brevibacterium* is not harmful when inhaled.
- PL73 *Brevibacterium* is not irritating to the skin and to the eyes.

As any feed containing protein, PL73 *Brevibacterium* is a potential sensitizer to the skin and by inhalation. As it will be delivered in bulk, information corresponding to the risk phrases R42/43 ('May cause sensitization by inhalation and skin contact') according to Directive 2001/59/EC will be provided through the product's MSDS and in the document accompanying the delivery.

Overall, it is concluded that PL73 *Brevibacterium* has a low acute toxicity. The product may be a sensitizer (risk phrase R42/43).

* Subchronic and genetic toxicology studies

Studies carried out with PL73 *Brevibacterium* lead to the following conclusions with regard to the biological consequences:

- PL73 *Brevibacterium* is not mutagenic or clastogenic.
- In a 13-week oral toxicity study in rats, PL73 *Brevibacterium* is tolerated without obvious signs of toxicity at dietary levels up to 8% (equivalent to 4.1 - 4.5 g/kg bw/d).

- No effects on reproduction are expected on the basis of the reproduction parameters, which were found to be normal in the subchronic feeding study in rats.

- The results of a developmental toxicity study in the rat indicated that no effects on development are to be expected from feeding PL73 *Brevibacterium* to pregnant animals up to dietary levels of 8%.

Target animals

Studies carried out with PL73 *Brevibacterium* lead to the following conclusions with regard to the biological consequences:

- From a tolerance study in cows it is shown that no effect on feed intake, concentrations of protein, fat and lactose in milk, blood parameters, blood cell counts and animal health was observed in animals fed for 4 weeks with diets containing 5, 10, 15 and 20% PL73 *Brevibacterium*. From this study it can be concluded that including PL73 *Brevibacterium* to a level of 15.8% during four weeks in dairy cow rations, has no effect on feed intake, milk production and concentrations of protein, fat and lactose in milk.

- No effects on fertility or reproduction in the target animals are to be expected from the intended use of PL73 *Brevibacterium* in animal feed on the basis of the fertility and fecundity parameters in experimental animal studies.

- No effects on microflora in the gastrointestinal tract, colonisation of pathogens in the GI tract, or increased antibiotic resistance are to be expected from the intended use of PL73 *Brevibacterium* in animal feed.

- No residues from heavy metals, pesticides, PAHs, PCBs, dioxins, and mycotoxins originating from the raw materials used in the manufacturing process of PL73 *Brevibacterium* or that may be formed during this one, are expected in edible commodities or excreta of animals fed this by-product.
  Very low residue levels of anti-foaming agents in edible products and excreta cannot be excluded. If any potential effects may result from them, they are part of the toxicological assessment of PL73 *Brevibacterium* and not considered of relevance.

Conclusion

Overall, it is concluded that, based on the studies performed on target and experimental animals as well as considering supplemental information and toxicological considerations, that the intended use of PL73 *Brevibacterium* in animal feed is not expected to result in undesirable biological consequences for the target animal. Ruminants can tolerate a maximum incorporation rate of PL73 *Brevibacterium* in the daily ration of 15%. Workers handling the product are advised to take protective measures, which are described in the Material Safety Data Sheet.

6.2 Risk assessment of newly expressed proteins

Except for the protein corresponding to the enzymatic activity allowing the resistance of strain SO317/pCABL to kanamycin, other proteins expressed, as a result of the construction of strain SO317/pCABL are proteins/enzymes of the general metabolism of *B. lactofermentum / C. glutamicum*. The risk assessment of these proteins is part of the overall assessment of PL73 *Brevibacterium*. Based on these data, proteins part of PL73 *Brevibacterium* are not considered to be of specific health relevance.

6.3 Testing of new constituents other than proteins

PL73 *Brevibacterium* is not known to contain new constituents. The constituents (other than proteins) present in PL73 *Brevibacterium* are found in a number of other feed materials. They were assessed within the framework of the different safety studies carried out on PL73 *Brevibacterium*. Based on these data, constituents that are part of PL73 *Brevibacterium* are not considered to be of health relevance.

6.4 Information on natural food and feed constituents

PL73 *Brevibacterium* is a complex product and its constituents are found in a number of other feed materials. As a new feed material without a conventional counterpart to which it could be compared to (even at least partially), PL73 *Brevibacterium* was assessed as such regarding its safety and nutritional value.

6.5 Testing of the whole GM product

The following studies were performed for the whole product PL73 *Brevibacterium*:
- acute toxicity studies
- (sub)chronic toxicity studies
- genetic toxicology testing
- studies on target species: cows and sheep
  * tolerance studies
  * performance studies
  * digestibility studies

The results are presented in C.6.1 and C.6.9.2 of this summary.

6.6 Allergenicity

As PL73 *Brevibacterium* is used in feed, it is noted that regarding animal health, allergenicity is not an issue that needs to be addressed specifically (section C.6.8 of EFSA guidance document).
Although allergenicity is not an issue for consumer safety in this respect, allergenicity/risk of sensitization was considered in the framework of workers safety. Any food/feed material containing proteins, including PL73 *Brevibacterium* may present a risk of skin or respiratory sensitization. PL73 *Brevibacterium* is therefore considered to be a potential sensitiser to the skin and by inhalation (risk phrase R42/43, see section C.6.1 Toxicology).

6.7 Assessment of allergenicity of newly expressed protein

According to the EFSA Guidance document, Section III.C.6.8 'Regarding animal health, allergenicity is not a significant issue that needs to be addressed specifically'.
Therefore, although PL73 *Brevibacterium* may contain newly expressed proteins as a result of the construction of L-lysine producer strain, as it is intended to be used as feed material only, this section was not specifically addressed.

6.8 Assessment of allergenicity of the whole GM product

As PL73 *Brevibacterium* is intended for use in feed, it is noted that regarding animal health, allergenicity is not an issue that needs to be addressed specifically.

6.9 Nutritional assessment

PL73 *Brevibacterium* is a microbial biomass with a high crude protein content (821-825 g/kg DM), which indicates the potential utility of the product as protein source in diets for monogastrics (e.g. pigs).

PL73 *Brevibacterium* can be included as a nitrogen source in diets of highly productive dairy cows up to a level of 10% of the basal diet at the expense of other commonly used ingredients, when adequately taking into account the chemical composition and the derived nutritive value of PL73 *Brevibacterium*. The optimum level of incorporation of PL73 *Brevibacterium* in the rations for dairy cows is up to 10%.
PL73 *Brevibacterium* should be regarded as a feed material for ruminants with a relatively low energy and protein value. Despite the high concentration of crude protein, the estimated amount of available true protein of PL73 *Brevibacterium* for ruminants was relatively small.

Feeding cows a diet supplemented with PL73 *Brevibacterium* up to a level of 20% does not result in significant alterations in the composition of the milk with regard to fat, protein, and lactose content. All the milk samples maintain their good smell and taste characteristics. There are no obvious off-flavours or defects.

6.10 Post-market monitoring of GM products

The applicant has the opinion that no post marketing monitoring of PL73 *Brevibacterium* is necessary for the following reasons;

- This product does not contain viable cells nor transferable DNA of the lysine-producing GMM (strain SO317/pCABL).

- The product is placed on the market as feed material only.

PL73 *Brevibacterium* is on the market as feed material since 1976 as a product obtained using conventional strains of *B. lactofermentum*, and since January 1998 as a product obtained using GM strains of *B. lactofermentum*, without any report of adverse effects.