SUMMARY OF APPLICATION FOR THE GM PRODUCT PL73 *E.COLI* (LYS) FOR FEED USE

A. GENERAL INFORMATION

1. Details of application

a) Member State of application France
b) Application number Not yet allocated to the Applicant at the time of the remittance of the dossier to the French competent authorities.
c) Name of the product (commercial and other names) -For the purpose of this dossier: PL73 *E.coli* (LYS) -Commercial name: PROT-AEL-L (However, subject to the confirmation that it may be acceptable as registered trade mark)
d) Date of acknowledgement of valid application Validity of the application to be established by EFSA.

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 ■
If *yes*, specify	

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 *E. coli* (LYS)', subject of the present application, consists of the dried killed cells of a genetically modified strain of *Escherichia coli* K-12, named strain N°10S, intended to be used for the production of L-lysine by fermentation of substrates of agricultural origin.

Strain N°10S consists of the recipient strain NVC578 and two plasmid vectors named: pS183dT and pAKID14. The purpose of the genetic modification is to increase the enzymatic activities of the L-lysine biosynthetic pathway and of the general metabolism pathway.

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

The product 'PL73 *E. coli* (LYS)' (dried killed bacterial biomass) mentioned in a) will be a by-product of the L-lysine manufacturing process using strain N°10S (NVC578/ pS183dT and pAKID14)

c) Intended use of the product and types of users

The product is intended to be used as a feed material, a source of crude protein, for compound feeding stuffs formulated for pigs (grower – finisher), fish (salmonids), and ruminants (inter alia dairy cow).

The product will be sold in pellet form and 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). It may also cause feelings of discomfort.

Storage
The product has to be stored at dry conditions in standard silos and kept away from ignition and heat sources

Use in compound feedingstuffs:

<u>* Pigs</u> (for fattening, grower – finisher).
Maximum incorporation rate in the feed: 6% (as is basis) pigs for fattening.

<u>* Dairy cows</u> (for milk production) <u>& ruminants</u> (in general for meat and milk production as from the beginning of rumination)
Maximum incorporation rate in the feed: 8% (as is basis or 7.3% DM basis) dairy cows.

<u>* Salmonids</u>
Maximum incorporation rate in the feed: 13% (or replacement of 20% of fish meal. Fish feeding stuff containing 65% fish meal).

e) Any proposed packaging requirements

No proposed packaging requirements, the product will be sold 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, produced from genetically modified micro-organisms
- Nitrogen expressed as crude protein
- Moisture: maximum 10%
- Crude Ash
- Approval number (Regulation 183/2005 -Directive 95/69/EC)

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, produced from genetically modified organisms'
-Amount of the product contained in food stuffs
-Percentage of the total crude protein provided by non-protein nitrogen

As the product will be delivered in bulk to feed mills (delivery by means of tank trucks), the information corresponding to labelling will be 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 N°10S will be used for production in containment only.
The manufacturing process of L-lysine and of the by-product / dried bacterial biomass 'PL73 E. coli (LYS) ensures that the final product will 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 authorization sought by the applicant for a use as feed material of the product 'PL73 *E. coli* (LYS)' concern the E.U internal 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 *E.coli* (LYS) does not contain viable cells and no 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 Strain NVC578
b) Strain designation Not applicable. Strain NVC578 is only a laboratory strain used for the construction of the final L-lysine producer microorganism (i.e. strain N°10S).
c) Source of the strain *E. coli* K12
d) Accession number from a recognized culture collection Strain NVC578 has not been deposited in a culture collection. It is only a laboratory strain used for the construction of the final L-lysine producer microorganism (i.e. strain N°10S).

1.2 Taxonomy

a) Genus *Escherichia*
b) Species *Escherichia Coli*
c) Subspecies Not applicable
d) Strain K12

1.3 Other names

There are no other names for the recipient strain.

1.4 Phenotypic and genetic markers

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

-Single cells, which are Gram-negative rods and not sporulating
-Colony size around 2 to 3 mm, round, rough and whitish with a clear edge
-Glucuronidase activity
-No evidence of instability of the recipient strain

b) Information on pathogenicity

In the performed studies, absence of pathogenicity was shown. The results of these studies are described in B.1.11.d of this summary.

1.5 Degree of relatedness between recipient and donor(s), when appropriate

Except two genetic modifications the sequences introduced or modified in the final strain N°10S are all coming from *E. coli* K12 genomes or vectors/ transposons developed from *E. coli* K12 strains.

1.6 Description of identification and detection techniques

Ribotyping and serotyping are used as detection techniques.

1.7 Sensitivity, reliability and specificity of the detection techniques

This is not relevant for the recipient strain, as it is a laboratory strain.

1.8 Source and natural habitat of the recipient microorganism

Not applicable for GM product falling within Group 2.

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

The recipient strain has neither conjugative plasmids nor self-transmissible plasmids. Therefore, the possibility of natural transfer is expected to be very low.

1.10 Information on the genetic stability of the recipient microorganism

No evidence of instability of the recipient strain.

1.11 Pathogenicity, ecological and physiological traits

a) Classification of hazard according to the current Community legislation

E. coli K12 strains are to be categorized in Group 1 according to Directive 2000/54/EC within the European Union. Microorganisms in this group are biological agents, which are unlikely to cause human disease.

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

E. coli has a doubling time of less than one hour, but this is strongly increased in the case of *E. coli* K12 strains producing amino acids (more than 2 hours). The mode of reproduction is the vegetative form.

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

E. coli K12 does not produce spores and has no other survival structures.

d) Pathogenicity

-*E. coli* K12 is listed as a non-pathogenic micro-organism
-Safety has been extensively reviewed and *E. coli* K12 is known as non-toxigenic

Study results:
-NVC578 (recipient strain) does not possess genes for pathogenicity factors: factor of adhesion, invasion, survival in tissues, cytotonicity or cytotoxicity (molecular typing study).
-Parental *E. coli* strains did not produce heat-labile enterotoxins, heat-stable enterotoxins or verotoxin or have pathogenicity factors.
-*E. coli* K12 strains have not been reported to cause allergic reactions

Test results allow the conclusion that *E. coli* K12 is not pathogenic to humans

e) Presence of genes that confer antibiotic resistance

The *E. coli* K12 strain is not expected to present antibiotic resistance.

f) Involvement in environmental processes

Not applicable, because this concerns an intermediate strain which is only used at laboratory level.

1.12 Information on indigenous mobile genetic elements

No presence of indigenous mobile elements was found in the parental *E. coli* K12 strain.

1.13 Description of its history of use

No reported instances of this micro-organism's pathogenicity or toxicity during its more than 50 years of laboratory use.

1.14 History of previous genetic modifications

The recipient strain NVC578 is obtained from *E. coli* K12 by several steps of genetic modification. This information is considered as confidential information. Therefore, no details are provided in the summary.

2. Characteristics of the donor organism(s)

This section is already described for *E. coli* K12 derivative strains in part B.1 of this summary. Here the characteristics of the other two donor organisms are described. However, some information of this section is considered as confidential information, like the names of these two donor organisms, and is therefore not provided in this (publicly available) summary.

2.1 Identity

a) Common name

The GM product, N°10S, consists of the recipient strain, NVC578, and two plasmid vectors named: pS183dT and pAKID14. The plasmid vectors are created from *E. coli* derivative strains and genes/ parts coming from two other microorganisms of which the names (at least for one) and some characteristics (for both) are considered as confidential information.

b) Strain designation

- 1st donor organism: *E. coli* H155
- 2nd donor organism: This information is considered as confidential information. Therefore, no information is provided in this summary.

c) Source of the strain

This information is considered as confidential information. Therefore, no information is provided in this summary.

d) Accession number from a recognized culture collection

The accession number, at least for the one out of the two donor organisms which has been deposited in a culture collection, is considered as confidential information and are, therefore, not included in this summary.

2.2 Taxonomy

Information about the taxonomy of the two donor microorganisms is considered as confidential information and left out of the summary. Information about the taxonomy of *E. coli* K12 derivative strains is already provided in section B.1.2 of this summary.

2.3 Other names

There are no other names for the donor microorganisms.

2.4 Phenotypic and genetic markers

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

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

b) Information on pathogenicity

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

2.5 Description of identification and detection techniques

It is not relevant to develop detection techniques for the donor strains (as it concerns laboratory strains). Only the amino acid producing strains are relevant in this respect.

2.6 Sensitivity, reliability and specificity of the detection techniques

It is not relevant to develop detection techniques for the donor strains (as it concerns laboratory strains). Only the amino acid producing strains are relevant in this respect.

2.7 Source and habitat of the organism

The section is not relevant because both donor organisms are laboratory strains.

2.8 Pathogenicity traits

For one donor organism the absence of any factor of adhesion, invasion, survival in tissues, cytotonicity or cytotoxicity was shown in studies. Therefore, it was concluded not to be pathogenic to humans.

The other donor organism is recognized as a non-pathogenic microorganism based on its long history of use and a literature search over more than 30 years.

2.9 Description of its history of use

For one of the donor organism, the applicant is not aware of information about this one, beyond its own usage of the strain.

The other donor organism has a long history of use.

3. Description of the genetic modification process

3.1 Characteristics of the vector

a) Nature and source of the vector N°10S is constructed of recipient strain NVC578 with two inserted plasmids, pS183dT and pAKID14. Both plasmids are constructed of *E. coli* K12 derivative strains and genes/ parts derived from two other organisms.
b) The copy number This information is considered as confidential and is, therefore, not included in this summary.
c) Description of the function of the main sequences inserted in the plasmid Each fragment inserted is well characterised and sequenced and does not contain unknown open reading frames. The plasmids do not present homologous sequences with mob and tra sequences. More specific information is considered as confidential and is, therefore, not included in this summary.

3.2 Information relating to the genetic modification

This information is considered as confidential 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

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

5.3 Stability of the microorganism in terms of genetic traits

The stability of the genetic modifications is analysed by three approaches:

- Lysine production ability by fermentation tests,
- Measurement of the percentage of colony forming units containing the two plasmids on minimum medium and complete medium containing kanamycine on cell suspensions after fermentation tests. For the two plasmids, the percentage of colony forming units containing the plasmids is close to 100%.
- Analysis of the plasmids extracted from cell suspensions after fermentation tests. The analysis of plasmids 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 plasmids, sine qua non conditions for a high lysine production.

5.4 Rate and level of expression of the new genetic material

Not applicable for GM product falling within Group 2

5.5 Description of identification and detection techniques

The traceability method proposed for 'PL73 *E. coli* (LYS)' could be applied to strain N°10S for its identification.

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

Except the Km gene present in one of the two plasmids present in strain N°10S and conferring resistance to kanamycin the other genes present in the plasmids are genes related to amino acid metabolism. However, the plasmid containing the Km gene was constructed from a non-conjugative plasmid and, as indicated in section B.3, the plasmids do not present homologous sequences with mob and tra sequences.

Moreover, an adequate inactivation process was developed and will be 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 producer microorganism in the final product 'PL73 *E. coli* (LYS)'.
The main risk for genetic transfer is related to DNA transformation. The DNA of strain N°10S is degraded extensively by the inactivation process and this results in the presence of non-functional DNA in the final product 'PL73 *E. coli* (LYS)'.

5.7 Information on the interaction of the GMM with other organisms

Not applicable for GM product falling within Group 2.

5.8 History of previous releases or uses of the GMM

Not applicable to the GMM. This microorganism has not been subject of previous releases/ uses.

5.9 Safety for humans and animals

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

No details on the genetic modification process are provided, because this concerns confidential information. It may be stated that considering the nature of the modifications made, the characteristics of the L-lysine / PL73 *E. coli* (LYS) manufacturing process no toxic, allergenic or other harmful effects on human or animal health are to be expected from the L-lysine producer microorganism N°10S.

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

This section is answered in section C.4 of this summary.

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

Not applicable to PL73 *E. coli* (LYS) deriving from the GMM as this bacterial biomass does not contain viable cells of the GMM.

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

Not applicable to PL73 *E. coli* (LYS) deriving from the GMM as this bacterial biomass does not contain viable cells of the GMM.

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.

C. INFORMATION RELATING TO THE GM PRODUCT

1. Information relating to the production process

PL73 *E.coli* (LYS) is a by-product of the L-Lysine fermentation process. L-lysine is produced by a fermentation process ('fed-batch fermentation') of a selected strain of *E. coli* K12 which has been modified to produce L-lysine. The fermentation culture medium consists of carbon sources, nitrogen sources, salts, amino acids and vitamins. The production of PL73 *E.coli* (LYS) consists of the following steps: strain preservation, culturing of seeds, fermentation using the sterilised raw materials of the fermentation culture medium, ammonia and filtrated air. Afterwards the broth is inactivated and subjected to further processing containing the following steps: 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.

2. Information relating to the product purification process

2.1 Technique used to remove microbial cells from the product

Not applicable since the killed microbial cells are not removed from the product. PL73 *E. coli* (LYS), in essence, contains inactivated and denatured microbial cells.

2.2 Information on the technique used to kill the microbial cells

The micro-organism inactivation procedure has been defined on the basis of bibliographic data on the sensitivity of *E. coli* to heat. As all vegetative cells, *E. coli* shows a high sensitivity to heat treatment, contrary to spore-forming organisms.

The possible presence of viable cells in PL73 *E. coli* (LYS) after the inactivation procedure is investigated by detection of bacterial growth of viable cells after plating on *E. coli* specific media. No viable PL73 *E. coli* (LYS) was detected in 3 grams of product tested.

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 really applicable to products such as PL73 *E. coli* (LYS).
Although, inactivated bacterial cells making up PL73 *E. coli* (LYS) are washed during their recovery.

3. Description of the product

3.1 Designation of the product

PL73 *E. coli* (LYS) is a dried killed bacterial biomass, a by-product of L-lysine production by fermentation using a genetically modified strain of *E. coli* K12. PL73 *E. coli* (LYS) is intended to be used as feed material. PL73 *E. coli* (LYS) has a complex nature and does not contain viable cells nor transferable DNA of the GMM. The proposed commercial name of PL73 *E.coli* (LYS) will be 'PROT-AEL-L' (standing for 'Protein-Ajinomoto Eurolysine- Lysine')

The product will be delivered in bulk to feed mills. The information corresponding to labelling will be provided to customers by means of the commercial documents preceding or accompanying the delivery of PL73 *E. coli* (LYS) and the commercial technical sheet corresponding to this product.

3.2 Intended use and mode of action

The product is intended to be used as a feed material supplying protein in compound feeding stuffs for pigs (grower-finisher), fish (salmonids), ruminants (in particular dairy cow).

3.3 Composition

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

*Nitrogen components (total and free amino acids, ammonium N, amide N, urea N, biogenic amines, 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

PL73 *E. coli* (LYS) has a high crude protein content (837 g/kg DM). Approximately 10% of the nitrogen is present in the form of ammonium-N in PL73 *E. coli* (LYS). The remaining part of the N-containing fraction consists mainly of true protein and amino acids (sum of amino acids after acid hydrolysis is 632 g/kg DM). The product also contains a substantial amount of sulphates (89 g/kg DM).
The content of PL73 *E. coli* (LYS) in crude fat, sugars / carbohydrates, and crude fiber is low or very low, respectively about 63g, 24g and 10g per kg DM.

3.4 Physical properties

PL73 *E. coli* (LYS) is a slightly brown product with a bulk density of 0.664 kg/L and a pH of 4.4.
-Particle size distribution: 10% (v/v): ≤ 10 µm
50% (v/v): ≤ 62 µm
90% (v/v): ≤ 140 µm
-Electrostatic properties (mJ): MIE[1] > 1200
-Auto-ignition: 540 °C
-Thermoanalysis: 247 °C (Classified as 'among most reactive dusts')
-Explosivity:
Pmax: 7.3 bar
MRPmax: 280 bar/s
Kst: 76 bar
Explosion class: St1

3.5 Technological properties

A large quantity of PL73 *E. coli* (LYS) – batch of about 20 tons - was produced for carrying all studies necessary to the application. To minimize any risk of degradation, especially microbial degradation, of this quantity of PL73 *E. coli* (LYS) until parts are taken to prepare the various experimental diets for the studies to evaluate its safety and nutritive value or for carrying out stability studies, it was stored in refrigerated conditions at 2-3°C with 70% humidity in big bags of about 1 ton each. This quantity was subject to regular monitoring and microbiological analyses over a total period of 23 months. PL73 *E.coli* (LYS) was stable from a microbiological perspective during the storage period of 23 months at 2-3 °C. Therefore, the chemical composition and the nutritive value of the product were not altered due to the activity of microorganisms during this storage.

The effects of different climatic conditions - combinations of different temperatures and relative humidity (RH) - on the behaviour and stability of PL73 *E. coli* (LYS), as such, or of compound feeding stuffs containing it (compound feeding stuffs prepared to evaluate its nutritive value) were investigated:
- PL73 *E. coli* (LYS) was chemically and physically stable during 12 months storage at 5 different climatic conditions, covering a wide range of moderate and subtropical conditions. PL73 *E. coli* (LYS) demonstrated a good microbiological quality during the storage period and can, therefore, be considered as microbiologically safe feed material.
- Pig feeds and dairy concentrates containing max. 20% PL73 *E. coli* (LYS) were chemically stable during 6 months storage at 3 different climatic conditions, covering a realistic range of moderate and subtropical 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

For PL73 *E. coli* (LYS) - when considering the construction of the L-lysine–producing strain N°10S resulting from the insertion of two plasmids pS183dt and pAKID14 (one of which containing the Km gene conferring kanamycin resistance to strain N°10S) - 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 resistance gene. In fact, except the Km gene, the other genes present in the plasmids are genes related to amino acid metabolism.

The inactivation procedure applied to the (fermentation) 'broth out' effectively kills the cells of the GMM, demonstrating eradication of viable cells from PL73 *E. coli* (LYS).

The potential presence of plasmid DNA in the product after inactivation was also identified and characterized. The presence of DNA in PL73 *E. coli* (LYS) was assessed by PCR (Polymerase Chain Reaction). It was concluded that the product contains low levels of pAKID14 plasmid fragments. However, these are small size fragments and do not contain the complete *npt*I gene. Therefore, gene transfer from the product is considered very unlikely. This is in accordance with the negative results obtained in the transformation experiments in which no marker gene resistant transformation was detected with the equivalent of 100 mg of product (20 transformation experiments, each carried out with the extracts corresponding to 5 mg of product).

5. Comparison of the GM product with its conventional counterpart

This section is not applicable to PL73 *E. coli* (LYS), intended to be placed on the market as feed material, because no biomass resulting from the production of L-lysine using a conventional strain of *E. coli* K12 has been previously manufactured and placed on the market. A comparative risk assessment with a conventional counterpart is therefore not possible. A full risk assessment was therefore carried out.

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

6.1 Toxicology

The toxicology of PL73 *E. coli* (LYS) 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 *E. coli* (LYS) in rats has an LD_{50} > 2000 mg/kg bw.
PL73 *E. coli* (LYS) is not harmful when ingested.

- The acute inhalation toxicity of PL73 *E. coli* (LYS) in rats has an LD_{50} > 5.26 g/m^3.
PL73 *E. coli* (LYS) is not harmful when inhaled.

- PL73 *E. coli* (LYS) is not irritating to the skin and to the eyes.

- As any feed containing protein, PL73 *E. coli* (LYS) is a potential sensitiser to the skin and by inhalation for employees handling it. 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 *E. coli* (LYS) has a low acute toxicity. The product may be a sensitiser (risk phrase R42/43).

Subchronic and genetic toxicology studies:

Studies carried out with PL73 *E. coli* (LYS) lead to the following conclusions with regard to the biological

consequences:

- PL73 *E. coli* (LYS) is not mutagenic.
- In a 13-week oral toxicity study in rats, PL73 *E. coli* (LYS) is tolerated without obvious signs of toxicity at dietary levels up to 15% (equivalent to 7.5-7.7 g/kg body weight/day).
- 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 indicate that no maternal and developmental effects are to be expected from feeding PL73 *E. coli* (LYS) to pregnant animals up to dietary levels of 15% (equivalent to 7.6-9.8 g/kg body weight/day).

Target animals

Studies carried out with PL73 *E. coli* (LYS) lead to the following conclusions with regard to the biological consequences:

- Cows can tolerate feed supplemented with up to 10% PL73 *E. coli* (LYS) without negative effects on feed intake and milk production and concentrations of protein, fat and lactose in milk.
- In pigs no specific toxic or detrimental effect of the use of PL73 *E. coli* (LYS) as dietary protein source is seen except for a lower faecal consistency. On the basis of the tolerance study it is concluded that PL73 *E. coli* (LYS) can be included in the diet of pigs (grower – finisher) as a protein source up to a level of at least 5% without effects on the zootechnical performances or health of pigs.
- No effects on fertility or reproduction in the target animals are to be expected from the intended use of PL73 *E. coli* (LYS) 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 *E. coli* (LYS) 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 *E. coli* (LYS) 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 agent in edible products and excreta cannot be excluded. If any potential effects may result from them, they were assessed through the different studies performed to assess the safety of PL73 *E. coli* (LYS).

It is noted that PL73 *E. coli* (LYS) is a complex mixture of components for which the following considerations comply: components other than proteins are usual components/ nutrients present in feed materials (of which metabolism is known), microbial strain L-lysine is well identified and characterised (does not produce toxins) and the product does not contain contaminants of toxicological concern at the levels present. From these points it was concluded that studies on the metabolism of PL73 *E. coli* (LYS) were not considered of additional value.

Conclusion

Overall, it is concluded that, based on studies performed on target and experimental animals as well as on supplemental information and toxicological considerations, the intended use of PL73 *E. coli* (LYS) in animal feed is not expected to result in undesirable biological consequences for target animals or the environment. Target animals can tolerate a maximum incorporation rate of PL73 *E. coli* (LYS) in the daily ration of 5 and 10% for pigs and cows, respectively. Workers handling the product are advised to take protective measures, which are described in the MSDS.

6.2 Risk assessment of newly expressed proteins

Except for the protein corresponding to the enzymatic activity allowing the resistance of strain N°10S to kanamycin, other proteins expressed, as a result of the construction of strain N°10S, are proteins/enzymes of the general metabolism of *E. coli*/*E. coli* K12 or of the metabolic pathways leading to lysine production and promoting it. The risk assessment of these proteins is part of the overall assessment of PL73 *E. coli* (LYS). Based on these data, proteins part of PL73 *E. coli* (LYS) are not considered to be of health relevance.

6.3 Testing of new constituents other than proteins

PL73 *E. coli* (LYS) is not known to contain new constituents.

6.4 Information on natural food and feed constituents

PL73 *E. coli* (LYS) is a complex product and its constituents, other than proteins, are found in a number of other feed materials. As a new feed material, without any conventional counterpart to which it could be compared to, PL73 *E. coli* (LYS) 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:
-acute toxicity studies
-subchronic toxicity studies
-genetic toxicology testing
-studies on target species: cows, pigs and fish
  *digestibility studies
  *tolerance studies
  *performance studies

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

6.6 Allergenicity

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

6.7 Assessment of allergenicity of newly expressed protein

According to EFSA Guidance document, Section III.C.6.8 ‘’Regarding animal healthy, allergenicity is not a significant issue that needs to be addressed specifically’’.
Therefore, although PL73 *E. coli* (LYS) may contain newly expressed proteins as a result of the construction of L-lysine producer strain N°10S, this section was not specifically addressed.

6.8 Assessment of allergenicity of the whole GM product

As PL73 *E. coli* (LYS) 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 of GM feed

PL73 *E. coli* (LYS) can be described as a microbial biomass with a high crude protein content (837 g/kg dry matter). Approximately 10% of the nitrogen is present in the form of ammonium-N. The remaining part of the N-containing fraction consists mainly of true protein and amino acids.

The in vitro digestibility study, in combination with the digestibility and performance studies in pigs indicates that PL73 *E. coli* (LYS) is a suitable protein source in the diet for pigs (monogastrics). The optimal nutritional level of the product PL73 *E. coli* (LYS) for pigs is up to a level of 60 g/kg (as is basis). The overall eating quality of meat from pigs fed with a diet containing PL73 *E. coli* (LYS) above this level is not found to be different from the control group fed a standard diet without the test product. The meat was only found to be slightly less tough in comparison with the control group.

The digestibility study in sheep and the performance study in cows indicate that PL73 *E. coli* (LYS) is a suitable protein source in the diet for ruminants. The optimal nutritional level of the product PL73 *E. coli* (LYS) for ruminants is up to a level of 73 g/kg DM (or ~800 g/kg on as is basis).

Feeding cows a diet supplemented with PL73 *E. coli* (LYS) 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 have good smell and taste characteristics. There are no obvious off-flavours nor defects.

The digestibility and growth studies in fish (rainbow trout) indicate that PL73 *E. coli* (LYS) is a suitable source of protein that can be substituted to fishmeal in trout diet. Substitution of fishmeal by up to 20% PL73 *E. coli* (LYS) does not alter growth performances of the trout nor the animal characteristics.

6.10 Post-market monitoring of GM products

The applicant has the opinion that no post marketing monitoring of PL73 *E.coli* (LYS) is necessary because the product does not contain viable cells, nor transferable DNA of,the L-lysine –producing GMM. The product is intended to be placed on the market as feed material.
A somewhat comparable dried killed bacterial biomass resulting from the production of lysine using *Corynebacterium glutamicum/Brevibacterium lactofermentum*(1) has been placed 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* (notified as 'existing product' according to article 20 (1) of Regulation (EC) 1829/2003 and listed in the Register of GM Foods and Feeds), without any report of whatsoever adverse effect.

(1) These are two names for the same species: *Brevibacterium lactofermentum* has been re-classified as *Corynbacterium glutamicum*