Part II. Summary

A. General Information

1. Details of application

a)	Member State of application Denmark
b)	Application number EFSA-GMO-RX-pMT742/pAK729
c)	Name of the product (commercial and other names) NOVO Yeast Cream (pAK729); in Danish *NOVO Gærfløde (pAK729)*
d)	Date of acknowledgement of valid application To be given by EFSA

2. Applicant

a)	Name of applicant Novo Nordisk A/S
b)	Address of applicant External environment, Dept. 1415, ILK, STA1 Sandtoften 9 DK-2820 Gentofte Denmark
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 difference from the applicant (Commission Decision 2004/204/EC Art 3(a)(ii)) Inge-Lise Kjærsig External environment, Dept. 1415, ILK, STA1 Sandtoften 9 DK-2820 Gentofte Denmark

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 ad 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 Regulation No. 1829/2003, Art. 20	

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 The GM microorganisms were notified under Reg. 1829/2003, Art. 20, §1 (b) and §4.	

6. ***Has the GM microorganism or derived product 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 ■

8. ***General description of the product***

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

The recipient organism is *Saccharoymyces cerevisiae* MT663.
The function of the genetic modification has been to enable the large-scale production of medicinal peptides by an organism as close to bakers' yeast as technically possible. Very few steps of genetic modification separate this strain from the QPS-approved *S. cerevisiae*.

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

The product to be marketed is a feed material for pigs of all categories (cf. Council Directive 96/25/EC on feed materials). The product is a heat-killed yeast biomass that is the residue, also called yeast cream, after the large-scale fermentation of the yeast to produce the commercial medicinal peptide (cf. Council Directive of 30 June 1982 on certain products used in animal nutrition, product group 1.2.1., the cells of which have been killed).

c) Intended use of the product and types of users

The intended use of NOVO Yeast Cream is as a wet-feed material. This feeding is carried out using wet-feeding systems, in which various feed items are mixed in a dosing and mixing plant to ensure a balanced diet. The users of NOVO Yeast Cream are pig farmers.

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

The pig farmer that receives NOVO Yeast Cream is instructed:

- to ensure stirring once an hour for 2 to 3 min, and, before sending to mixing tank to make wet feed, to ensure stirring for 3 min., and
- to give the animals free access to water due to the feed material's high content of chloride, which derives from pH adjustment during Novo Nordisk' production process

There are no particular recommendations for handling, because the yeast cream is liquid and, thus, not dust-forming.

e) Any proposed packaging requirements

NOVO Yeast Cream is transported by lorry tankers directly from the Novo Nordisk production facility to the pig farmer's feed containers.

f) A proposal for labelling in accordance with Articles 13 and Articles 24 of Regulation (EC) 1829/2003. In the case of GMOs, food and/or feed containing or consisting of GMOs, a proposal for labelling 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

Each delivery of NOVO Yeast Cream is accompanied by a delivery note to the farmer. The delivery note, which is in Danish, has the following wording:

"Name: NOVO Yeast Cream. The product is a vegetable industrial by-product based on genetically modified strains of *Saccharomyces cerevisiae* (bakers' yeast). The yeast cells have been killed by heat-treatment. The product is produced by Novo Nordisk A/S, Hallas Allé 1, 4400 Kalundborg."

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

As there is no living GMO in NOVO Yeast Cream, a unique identifier is not relevant.

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.

There are no geographical limitations sought for NOVO Yeast Cream, and there are no types of environment that are unsuited for its use.

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

In case of spillage of NOVO Yeast Cream, the farmer and/or the driver of the lorry shall first of all take measures to prevent flow of the material to rivers and drains. Next, they are recommended to contact the Novo Nordisk-approved trader for further instructions.

B. INFORMATION RELATING TO THE GMM

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

1.1. Identity

Common name
GM bakers' yeast

a) Strain designation
S. cerevisiae MT663

b) Source of the strain
Novo Nordisk's strain collection

c)	Accession number from a recognised culture collection MT663: ATCC 20893

1.2 Taxonomy

a)	Genus *Saccharomyces*
b)	Species *cerevisiae*
c)	Subspecies Not relevant
d)	Strain MT663

1.3 Other names

a)	Generic name None
b)	Commercial name None
c)	Previous name(s) None

1.4 Phenotypic and genetic markers

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

Phenotypic and genotypic markers and sources of strains of *Saccharomyces cerevisiae* that are recipient strain in the dossier and its progenitors.

Strain name	Relevant phenotype	Genotype	Source	ATCC accession no.
S288C	QPS, haploid	*MATα SUC2 gal2 mal mel flo1 flo8-1 hap1 ho bio1 bio6*	Strain collection	204508
X2180-1A	QPS, haploid	*MAT**a** SUC2 CUP1 mal mel gal2*	Strain collection	204504
MT663	[A] Diploid, Tpi$^-$, Pep4$^-$	*MAT**a**/α leu2/leu2 HIS4/his4 pep4-3/pep4-3 Δtpi::LEU2/Δtpi::LEU2 Cir$^+$*	Developed at Novo Nordisk A/S from X2180-1A and S288C	20893

Abbreviations: QPS, qualified presumption of safety. Tpi$^-$, lacks triose phosphate isomerase (cannot grow on hexose sugars). Pep4$^-$, deficient in peptidase 4 (proteinase A). Yap$^-$, deficient in aspartic peptidase 3.
[A] Relative to strains X2180-1A and S288C, only Tpi$^-$ has been affected by genetic modification.

b) Information on pathogenicity
None of these strains is pathogenic, and both recipient strains are derived from the two QPS strains (S288C and X2180-1A).

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

The recipient is not related to donor. The former is yeast, and the latter is synthetic DNA sequences coding for a human insulin precursor.

1.6 Description of identification and detection techniques

There are clear phenotypical and genotypical differences between the recipient and its QPS progenitors. The recipient cannot grow on traditional yeast media with hexose sugars as the sole source of carbon because its gene for the glycolytic enzyme triose phosphate isomerase (*tpi1*) is interrupted. A PCR technique, deposited with the Community Reference Laboratory for GM Food and Feed, is also used to differentiate between wild-types and the GM strain. The PCR method amplifies the recipient strain's unique configuration of the *tpi1* gene with the *LEU*2 gene as an insertion in the middle of *tpi1*.

1.7 Sensitivity, reliability and specificicity of the detection techniques

The phenotypic detection technique that uses plating on the yeast media mentioned above is reliable and gives clear results. The PCR technique is also very reliable and specific. Discrimination between recipient and wild-type was very clear, as the two PCR products are 2920 and 1280 base pairs, respectively, giving a size difference of 1640 base pairs. These 3 sizes are clearly distinguishable with standard genetic techniques.

1.8 Source and natural habitat of the recipient microorganism

Not applicable as recipient is a direct derivate of the QPS *S. cerevisiae.*

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

Under natural conditions, only haploid cells of *S. cerevisiae* can transfer genetic material, and the recipient MT663 is diploid . The steps of genetic modification used in its derivation from the QPS strains have not involved any DNA of mobile elements.

1.10 Information on the genetic stability of the recipient microorganism

There is no indication of genetic instability of the recipient strain.

1.11 Pathogenicity, ecological and physiological traits

The recipient of this dossier derives from QPS strains of *S. cerevisiae*. The steps in genetic modification from the QPS strains to the recipient strain do not relate to any pathological or ecological traits. Of physiological traits, relative to the QPS progenitors, the steps of genetic

modification have only affected one trait in the recipient. The ability to synthesize the enzyme triose phosphate isomerase has been deleted.

a)	Classification of hazard according to the current Community legislation According to EU Directive 2000/54 on risks related to biological agents, the species *Saccharomyces cerevisiae* is categorized in Group 1, for biological agents that are unlikely to cause human disease. In addition, the species has been given the European Food Safety Authority status as QPS (qualified presumption of safety). Therefore, the sections immediately below are not relevant.
b)	Information on the doubling time and of the mode of reproduction
c)	Information on survival, ability to form spores or other survival structures
d)	Infectivity
e)	Toxigenicity
f)	Virulence
g)	Allergenicity
h)	Information on viability and ability to survive in the gastrointestinal tract of humans or animals
i)	Probiotics or immunomodulatory properties
j)	Presence of genes that confer antibiotic resistance
k)	Involvement in environmental processes

1.12 Information on indigenous mobile genetic elements

None of the GM steps that brought about the recipients have affected any indigenous mobile elements of the QPS progenitors.

1.13 Description of its history of use

Since 1989 the recipient strain *S. cerevisiae* MT663 has been in use for the same purpose and under the same conditions as applied for in this application. For these 20 years, the strain has been the host of insulin precursor plasmids and has been used in the expression of many other pharmaceutical proteins/peptides for investigational purposes since 1985. Since 1989, a very large part of the World's man-made insulin has been produced by this recipient, and all that yeast cream has gone to pigs as feed. The number of pigs that have eaten the enormous quantities of MT663 yeast cream is well over 5 million.

1.14 History of previous genetic modifications

The recipient organism of this dossier MT663is the host of the medicinal peptide production plasmid. The recipient strain was developed from QPS strains of *S. cerevisiae* through a series of classical genetic crosses and a few genetic modifications. Relative to the QPSprogenitors, the steps of genetic modification have only affected one trait in the recipient; i.e, the ability to synthesize the enzyme triose phosphate isomerase has been removed.To do this, rDNA techniques were used to inactivate the natural *S. cerevisiae* gene for its triose phosphate isomerase (*TPI1*). The inactivation was achieved by placing the yeast *LEU2* gene within the DNA of *TPI1*.

2. Characteristics of the donor organism(s)

The DNA encoding the sequence of the precursor for the commercial medicinal peptide has been exclusively made using synthetic oligonucleotides and standard techniques of molecular biology. Therefore, *per se* there is no donor organism. The basis of the knowledge of the DNA sequence is the publically accessible databases that contain the sequences of the human insulin. For the peptide in this dossier, the synthetic coding sequence is 176 nucleotides long, plus the yeast expression signals. The DNA sequence has been optimized using codons favourable for expression in *Saccharomyces cerevisiae*.

2.1. Identity

d)	Common name Not applicable
e)	Strain designation Not applicable
f)	Source of the strain Not applicable
g)	Accession number from a recognised culture collection Not applicable

2.2 Taxonomy
Not applicable

e)	Genus Not applicable
f)	Species Not applicable
g)	Subspecies
h)	Strain

2.3 Other names

d)	Generic name Not applicable
e)	Commercial name
f)	Previous names

2.4 Phenotypic and genetic markers
Not applicable

c)	Phenotypic and genotypic information relevant to identification, genetic stability and safety
d)	Information on pathogenicity

2.5 Description of identification and detection techniques

Not applicable

2.6. Sensitivity, reliability and specificity of the detection techniques

Not applicable

2.7 Source and natural habitat of the organism

Not applicable

2.8 Pathogenicity traits
Not applicable

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 pathotenicity islands

2.9 **Description of its history of use**

Not applicable

3. Description of the genetic modification process

This application relates to an expression plasmid used for the production of a medicinal peptide. The expression plasmid is part of a European Union registration file for medicinal products, some of which are marketed world-wide. The plasmid is:

pAK729.6.16D

The expression plasmid is genetically modified for the production of a precursor of the insulin molecule.

3.1 Characteristics of the vector

a) Nature and source of the vector

All vector DNA is from the QPS-approved yeast species *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* and from *E. coli* K-12 plasmid systems (EK2), that are exempt from the USA NIH Guidelines.

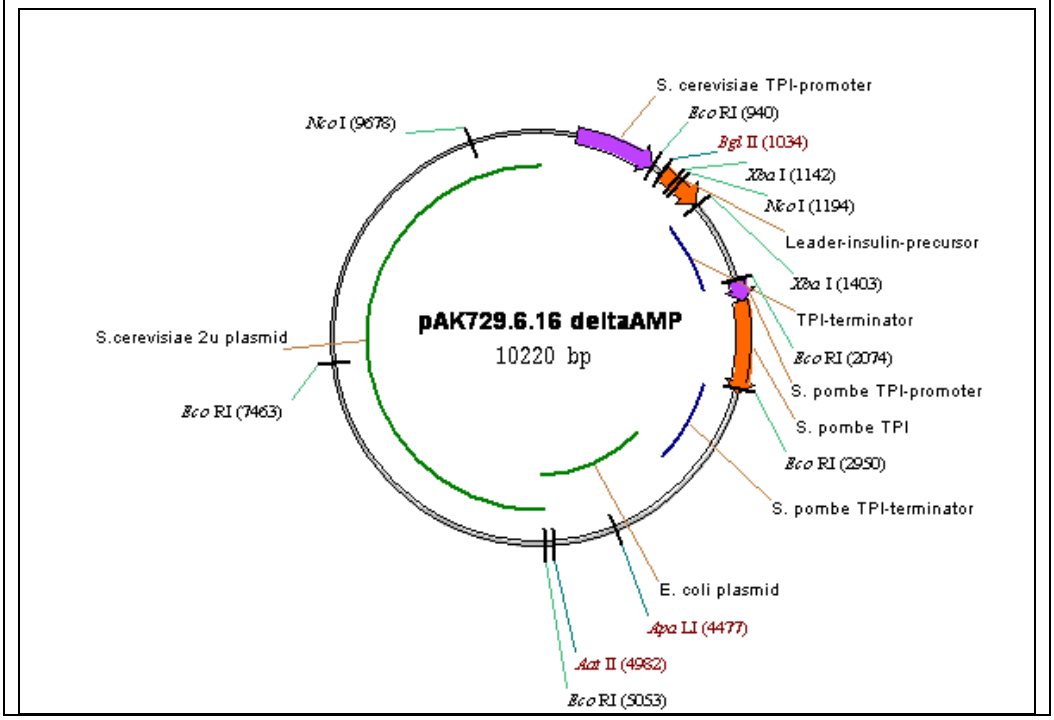
The vector itself is the basis of several expression plasmids at Novo Nordisk used for the production of different medicinal peptides. Into the basic vector, interchangeable cassettes are placed with the nucleotide sequences of the appropriate pre-pro form of the medicinal peptide and of the adjacent signal peptide amino acide sequence.

b) The copy number

Approximately that of the natural *S. cerevisiae* 2 µm-plasmid, i.e., 50.

c) Physical and genetic map

Below is the map of the expression plasmid in the dossier.



d) Position of probes and primers used

Information found in medicinal MAA (Marketing Authorisation Application) files.

e) Identification and description of each component

Component	Source	Function
Transcription promoter	*S. cerevisiae TPI1* (triose phosphate isomerase) gene	Necessary for start of transcription
DNA for leader peptide	*S. cerevisiae* MFα(1) gene or synthetic DNA	Leader directs and facilitates secretion of precursor of specific medicinal peptide
DNA for precursor of medicinal peptide	Synthetic DNA	Encodes amino acid sequence of precursor of insulin
Transcription terminator	*S. cerevisiae TPI* gene	Stops elongation of gene transcript
POT	*Schizosaccharomyces pombe TPI* gene	Gene for enzyme triose phosphate isomerase, essential for growth on medium containing glucose as sole carbon source
Ori for replication of *E. coli* plasmid	*E. coli* plasmid pUC13	Drives replication when plasmid is in *E. coli*
2µm-plasmid, major portion	*S. cerevisiae* plasmid	When plasmid in *S. cerevisiae*, drives replication, amplification, and inheritable stability of expression vector

f) Frequency of mobilisation of the vector and its capacity for genetic transfer

Vector cannot be mobilized because of *S. cerevisiae*'s characteristics of mating, classical genetic changes made to the host, and lack of sequences in vector for recombination.

g) Information relating to the host range of plasmid used as a vector

The vector is constructed as an *S. cerevisiae – E. coli* shuttle vector, but transfer between these two species can only occur as a result of transformation with purified DNA in the laboratory and specific selection for transformants.

3.2 Information relating to the genetic modification

a) Methods used to construct and introduce the insert(s) into the recipient or to delete a sequence(s)

These methods were both classical yeast genetic techniques and standard recombinant DNA techniques.

b) Integration site, sequence actually inserted or deleted, size and copy number of all detectable inserts Information in medicinal MAA (Marketing Authorisation Application) files.
c) Methods used for their detection The MAA's that encompass the expression plasmid contain a detection method to discriminate between the production strain (containing this plasmid) and contaminating strains of *S. cerevisiae*. The technique is validated and exploits the ability of the yeast cells to produce the medicinal product in question while growing on agar plates. The colonies are transferred to a nitrocellulose membrane, and the gene product is detected by antibodies produced against it.
d) Size and function of the deleted region(s) Information found in medicinal MAA files.
e) Purity of the insert Information found in medicinal MAA files.
f) Sequence of flanking regions Information found in medicinal MAA files.
g) Methods and criteria used for selection The expression plasmid can be selected for in the yeast cell due to the plasmid coding for the key enzyme in glycolysis triose phosphate isomerase. Cells without the plasmid grow very poorly on media with hexose sugars as the sole carbon source.
h) Subcellular location(s) of insert(s) Only in expression vector plasmid DNA, in cytoplasm.

4. Identification of the conventional counterpart microorganism and its characteristics

The conventional counterpart microorganism is *S. cerevisiae* X2180-1A, which is a wild-type strain of bakers' yeast. Wild-type strains of this species have the EFSA status of QPS. Strain X2180-1A can be traced back at least as far as to the 1930'ies and is itself the progenitor of the Novo Nordisk host strain in this application. The strain acted as a control organism in the 3 toxicological studies presented in the dossier.

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 GMM strain of *S. cerevisiae* in this application is YAK729.6 and carries the expression plasmid pAK729.6.16D.

The purpose of the genetic modifications that resulted in *S. cerevisiae* YAK729.6 was to produce a strain of *S. cerevisiae* for the large-scale production of a precursor of human insulin. Relative to its predecessor MT748, this strain expresses the insulin precursor more efficiently, and it lack the gene for ampicillin resistance.

There are no indications that the central metabolism of this strain is changed relative to its QPS predecessor. The triose phosphate isomerase gene that is inactivated in the recipient is restored in the GM strain due to its carriage on the plasmid.

The dossier contains a thorough analysis of the single unknown open reading frame that exists on the expression plasmid. Due to a number of biological and technological factors, it is extremely unlikely that the reading frame can have any consequences.

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

The expression plasmid remains in the final construction. Almost all this DNA is from QPS organisms. See Section 3 above.

5.3 Stability of the microorganism in terms of genetic traits

The GMM in the dossier is instrumental in commercial large-scale fermentations to produce the medicinal peptide; the genetic stability of the organism is crucial in this respect and has been ensured. After the large-scale fermentation, the GMM is heat-killed, why genetic stability after that stage is not relevant.

5.4 Rate and level of expression of the new genetic material

The biosynthesis, structure, and biochemical properties of insulin are all very well characterized and are part of the registration files for the medicinal products.

It is to be noted that because the yeast cells in the yeast cream as pig feed have been killed, no expression of any yeast genes takes place in the feed product.

5.5 Description of identification and detection techniques

The MAA (Marketing Authorisation Application) covering the medicinal products from the GMM *S. cerevisiae* contains a detection method to discriminate between the production strain (containing the plasmid) and contaminating strains of *S. cerevisiae*. The technique is validated and exploits the ability of the yeast cells to produce the medicinal product in question while growing on agar plates. The colonies are transferred to a nitrocellulose membrane, and the gene product is detected by antibodies produced against it.

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

he cells in the yeast cream pig feed are dead so that no form of cell-mediated transfer of genetic material from the yeast can take place. The heat treatment at about 80°C, that kills the cells, also leads to extensive fragmentation of the cells' DNA, including the recombinant DNA. The subsequent fermentation of the yeast cream with the silage culture leads to further degradation of the genetic material. Thus, it is virtually impossible that any uptake and expression of recombinant DNA can take place from the dead yeast cells into other living cells.

5.7 Information on the interaction of the GMM with other organisms

The yeast cells in the yeast cream have been very effectively heat-killed and, thus, cannot biologically interact with other organisms.

5.8 History of previous releases or uses of the GMM

Yeast cream from the strain YAK729.6 has been used for this purpose, i.e., as an ingredient in pig feed, since 2004. Except for the production of the medicinal peptide, this strain has not been used for any other purpose.

5.9 Safety for humans and animals

a) Information on any toxic, allergenic or other harmful effects on human or animal health Yeast cream from this GMM strain has recently been subjected to a 90-day feeding trial in rats, to an Ames test and to an *in vitro* chromosomal aberration test. No effects were seen that were not present in the QPS yeast cream, and those effects were only due to high salt in the very high dose level in the rats.
b) Potential for DNA transfer or any capacity for enhanced gene transfer The cells in the yeast cream pig feed are dead so that no form of cell-mediated transfer of genetic material from the yeast can take place. The heat treatment at 80°C that kills the cells ((See Section C.1. below) also leads to extensive fragmentation of the cells' DNA, including the recombinant DNA. The subsequent fermentation of the yeast cream with the silage culture leads to further degradation of the genetic material. Thus, it is virtually impossible that any uptake and expression of recombinant DNA can take place from the dead yeast cells into other living cells.
c) Viability and residence time of the GMM in the alimentary tract The GMM is dead, and residence time is not different from dead cells of the conventional counterpart.
d) Information on any impact of the GMM on the microbiota of the human or animal gastrointestinal tract The GMM yeast in the yeast cream is dead, so that they cannot impact the microbiota of the GI tract any differently from a QPS yeast, the conventional counterpart.

5.10 Information on monitoring control waste treatment and emergency response plans

Not relevant as the yeast are dead.

C. INFORMATION RELATING TO THE GM PRODUCT

The GM product of this dossier is a feed material whose environmental sustainability is noteworthy. The feed material constitutes the re-use of a residue of another industrial production, and as additional raw materials only makes use of a silage culture and a sugar solution for that culture. In addition, the product is exceptionally nutritional for the animal and is only transported short distances to the pig farms from its point of production.

1. Information relating to the production process

The yeast cream product is a residue after the commercial manufacture of a precursor of the insulin molecule. It is produced during fermentation by bakers' yeast that has been genetically modified. After the large-scale fermentation of the yeast, the precursor is extracted from the yeast biomass. During the fermentation and purification of the precursors, the pH is adjusted, mostly with HCl or NaOH. The residual biomass is then heat treated at about 80°C, which

reduces the viable count of the yeast cells from about 10E10 CFU/ml to below the detection level of ten CFU/ml. This heat also extensively fragments the DNA. Finally, a commercial, EU-approved silage culture, i.e., lactic acid bacteria, and a sugar solution, are added to the biomass in order to preserve the yeast cream. The yeast cream is transported in lorries directly from Novo Nordisk to the pig farms.

2. Information relating to the product purification process

2.1 Technique used to remove microbial cells from the product

Instead of removing the microbial cells from the product, the product is heat-treated, which kills all yeast cells. The efficacy of the killing is routinely monitored by Novo Nordisk.

2.2. Information on the technique used to kill the microbial cells

The yeast cells are heat-killed at 80°C.

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

During the manufacture of the medicinal product after the yeast fermentation, the yeast cells are centrifuged and washed in buffer as part of the process of harvesting the medicinal peptide that the yeast produce. Thus, the resulting feed material is yeast biomass, which is partially purified from the yeast growth medium. The growth medium is virtually identical to that used to grow *S. cerevisiae* for research purposes, consisting of yeast extract, carbohydrates, vitamins, and minerals.

3. Description of the product

3.1 Designation of the product

The feed to which this application relates is traded under the designation NOVO Yeast Cream (pAK729), or in Danish "NOVO Gærfløde (pAK729)." There are no other synonyms for the product.

NOVO Yeast Cream from GM yeast has been marketed in Denmark for 20 years, and its use is well established. Each delivery is followed by a traceable number, and the product can only be purchased through a Novo Nordisk-approved trader.

According to European Union Directive 82/471/EEC on certain products used in animal nutrition, the yeast cream belongs to the product category 1.2.1, as specified in the Annex of that directive; i.e., *Yeast cultivated on substrates of animal or vegetable origin, the cells of which have been killed* (European Council, 1982). The GM yeast is not removed from the product but is heat-killed. The product is not purified in the sense specified in the EFSA *Guidance document* (European Food Safety Authority, 2006).

3.2 Intended use and mode of action

The intended use of NOVO Yeast Cream is as a wet-feed material. The feeding is carried out using wet-feeding systems, in which various feed items are mixed in a dosing and mixing plant to ensure a balanced diet.

Wet-feeding is a widespread practice in Danish pig production. An advantage with the wet feeding system is that the farmer can reduce the cost by using home-produced, or domestic, starch-rich grains mixed with different byproducts high in protein e.g. yeast cream.

The recommended maximal concentration of yeast cream for different categories of pigs is shown in the table below.

Recommended maximal concentration of yeast cream in pig feed for different categories of the animal.

Animal category	**% in diet (on wet basis)**
Sows for reproduction	15
Sows, in order to have benefit in piglets	15
Piglets 7-30 kg	10
Piglets, suckling and weaned	20
Pigs for fattening	25

3.3 Composition

The NOVO Yeast Cream consists of the heat-killed yeast, salt from the process to purify the medicinal peptides, a commercial silage culture, and sugar for the silage culture's growth. An overview of the chemical composition is as shown in the table.

Overview of chemical composition of NOVO Yeast Cream.	
Parameter	**Content**
Dry matter (%)	14.7
100% dry matter	
Crude protein (%)	50.4
Crude fat (%)	4.1
Crude fiber (%)	1.9
Ash (%)	9.7
Calcium (%)	0.29
Sodium (%)	0.78
Chloride (%)	3.00
Phosphorus (total) (g/kg)	1.67

3.4 Physical properties

NOVO Yeast Cream is a thick, white liquid with a characteristic nutty smell. The dry matter content can vary from delivery to delivery, being 14 - 16%. The yeast cream will separate when stored. Storage tanks are therefore equipped with a stirring device. The shelf life of yeast cream is a least 14 days from delivery. At delivery the pH is 3.7 to 4.7. Normally the yeast cream is delivered to the farmers once every week.

3.5 Technological properties

The product is wet and thus does not form dust. NOVO Yeast Cream is stable when stored for up to 2 weeks. After 2 weeks, the continued fermentation in the product may result in a loss of dry matter and soluble nutrients and thus lower nutritional value.

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

Novo Nordisk has investigated the degradation in the yeast cream of the DNA of 4 different recombinant expression plasmids in 4 production strains, one of which is the object of this dossier. Using PCR techniques, the study showed that the severe heat treatment coupled with the growth of the silage bacteria led to very extensive DNA degradation. As one of the controls in the study, the QPS counterpart of the GM strains *S. cerevisiae* X2180-1A was treated in parallel with the 4 GM production strains. As virtually all strains of *S. cerevisiae*, the QPS counterpart naturally contains a large plasmid called the 2-micron plasmid, which also during development had been chosen as part of the backbone of the recombinant expression

plasmids. Thus, in this investigation of all five organisms, the status of the plasmid DNA was followed

- in pure cultures,
- in the yeast slurry heat-treated immediately after harvest, and finally
- in the finished feed material, i.e., in the yeast cream.

The study followed the status of the plasmid DNA both by simple agarose gel electrophoresis and by Southern blotting of the total plasmid DNA. The template for the probe for the blotting was each expression plasmid, including the one in this dossier. Such a probe will also detect the QPS DNA due to each vector also containing the 2-micron plasmid. For each investigation, the DNA was both unrestricted by endonucleases and restricted by the enzyme *Eco*RI.

The results of the investigation showed that heat treatment at high temperature and low pH results in efficient DNA degradation. The subsequent fermentation step with silage bacteria leads to yet further DNA decay. Very extensive DNA degradation was seen for YAK729.6 and for the QPS yeast, following their heat treatment at 80 – 85°C, coupled with the silage fermentation.

In 1985 Novo Nordisk investigated the ability of its production strains of *S. cerevisiae* to transfer genes to *E. coli*. Neither conjugation nor transformation of a resistance marker gene from the yeast to the bacterium could be detected. By natural means, the Novo Nordisk GM yeast strain cannot either transfer its genes to other yeast cells because the strain is diploid and thus incapable of mating with other cells.

5. Comparison of the GM product with its conventional counterpart

The conventional counterpart of the NOVO Yeast Cream as animal feed is of two types:

A. viable cells of the yeast species *Saccharomyces cerevisiae* and
B. the non-viable biomass residue of the same species after a yeast-driven biotechnological production.

Ad A. In the European Union there is a number of approved feed additives that contain *S. cerevisiae* as their active substance. These feed additives are in the categories gut flora stabilizers (category 4 (b)), silage additives (category 1(k), and a flavoring compound (category 2 (b)) (2009). The yeast species carries the EFSA status of QPS, and for pigs for fattening the viable organism may be added up to 10^{10} colony forming units per kg complete feed (2008).

Ad B. NOVO Yeast Cream resembles very closely yeast biomass that results from the yeast-driven industrial production of ethanol, in which the yeast are heat-killed after the process and then used as feed. The table below illustrates this resemblance, where the key components of NOVO Yeast Cream are compared with those in 3 other commercial yeast feed products, all also marketed in the European Union.

Comparison of key components in NOVO Yeast Cream and with those in three conventional counterparts, produced by Lantmännen AB, Lallemand, and Diamond V. Concentrations of the listed components have been converted to concentrations for a product of 100% dry matter.

	Yeast feed product, commercial producer			
Parameter	**Novo Nordisk's yeast cream (A)**	**Agrodrank 27, Lantmännen (B)**	**Spirits yeast cream, Lallemand (C)**	**Diamond V's Diamond V XPC (D)**
Dry matter (%)	14.7%	28%	23.8%	89%
	100% dry matter			
Crude protein	50.4	33.9	45.7	16.9
Crude fat	4.1	5.0	5.7	1.7
Crude fiber	1.9	1.6	0	24.7
Ash	9.7	7.5	9.6	9.0
Phosphorus (total)	1.67	1.36	0.56	0.76

Source:

A. (Danish Pig Production, 2006a)

B. (Lantmännen Agroetanol AB, 2008) (http://www.agroetanol.se/)

C. (Danish Pig Production, 2006b)

D. (Diamond V, 2009)

The dossier's comparative risk assessment shows that the composition of the Novo Nordisk product does not give rise to concern.

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

6.1 Toxicity

Three toxicological tests have recently been carried out to evaluate the potential impact of NOVO Yeast Cream from 4 GM strains on human and animal health. One of the 4 strains is YAK729, the object of this dossier. Firstly, a 90-day feeding trial in rats showed no adverse effects other than those also seen for the QPS control yeast cream. Here, when the yeast cream made up 40% dry weight of the rats' diet, which is approximately a 10-times overdose, the resulting high salt concentration caused some damage to the kidneys. It is concluded that the NOVO Yeast Cream is non-toxic in the 90-day feeding trial.

The two other toxicological studies were the Ames test for mutagenicity and an *in vitro* chromosome aberration test. In neither of these studies showed any adverse effect observed that was caused by the GM yeast cream, which therefore is non-genotoxic. Thus, it has been demonstrated that NOVO Yeast Cream from none of the 4 GM yeast tested poses any concern for human or animal health. The officially recommended free access to water lets the animal compensate for the extra intake of salt. Overall therefore, it is concluded that the intended use of yeast cream as animal feed cannot be expected to result in any undesirable biological effects for the target animal, for the consumer, or the worker handling the yeast cream.

Finally, it can be noted that NOVO Yeast Cream has been marketed to Danish pig farmers for more than 20 years, and there have never been any complaints from the farmers about any health effect for man nor animal that could have been caused by the yeast cream as such.

6.2 Risk assessment of newly expressed proteins

The dossier limits its risk assessment of proteins to those not found in QPS strains of *S. cerevisiae*. The section on toxicology immediately above describes the absence of any adverse effects from the NOVO Yeast Cream produced from 4 GM yeast strains, one of which is YAK729.

It can be noted that each of the 4 GM yeast strains produces its own medicinal peptide, and these peptides are removed from the fermentation broth. After fermentation, this peptide is extracted out of the fermentation medium in a very efficient and controlled process. Before being released as feed material, the yeast slurry is heat-treated and ensiled. The insulin precursor from the GM strain is not insulin itself. Furthermore, insulin itself would never function following oral ingestion in a feed due to digestive proteolysis.

The dossier contains a thorough analysis of the single unknown open reading frame that exists on the expression plasmid. Due to a number of biological and technological factors, it is extremely unlikely that the reading frame can have any consequences.

6.3 Testing of new constituents other than proteins

Relative to QPS strains of *S. cerevisiae*, the GM yeast strain in the NOVO Yeast Cream does not produce any new non-protein substances. The toxicological studies referred to above demonstrate this absence of toxic components.

6.4 Information on natural food and feed constituents

Relative to its conventional counterpart, there are no differences in the yeast biomass as a feed material. The following considerations explain this:

- Components produced other than proteins are usual components/nutrients present in feed materials.
- The levels of chloride, which are high in the yeast cream, are not the result of the genetic modification, but rather of the production process, in which the pH is adjusted, most often using NaOH and then HCl.
- The yeast strains are well defined and very well characterized and do not produce any new or altered levels of metabolites.
- As a result of toxicological testing, the constituents of the genetically modified yeast cream are considered not to pose any toxicological concern in relation to safety and nutritional value when compared to its conventional counterpart.

6.5 Testing of the whole GM product

Three toxicological studies have been performed on 4 GM yeast creams in comparison with yeast cream from the conventional counterpart and non-GM, QPS yeast strain *S. cerevisiae* X2180.1A. Yeast cream from strain YAK729 of this application was one of the 4 GM yeast creams in the study. The three toxicity studies are:

- 90-day rat feeding trial
- Ames test for mutagenicity
- *in vitro* chromosome aberration test

See Section 6.1. above for details. Based on the results from the 2 genotoxicity tests, it is concluded that yeast cream from the 4 GM yeasts is non-mutagenic and non-clastogenic as is their conventional counterpart, (i.e., yeast cream from the QPS yeast). In the 90-day feeding trial no adverse effects different from those observed in the QPS yeast cream were seen at the high dose-level of 40% or at the low-dose level of 15%.

Overall, it is concluded that the intended use of yeast cream in animal feed cannot be expected to result in any undesirable biological effects for the target animal, for the consumer or the worker handling the yeast cream.

6.6 Allergenicity

Just as for the QPS yeast cream, NOVO Yeast Cream from the GM yeast strain in this dossier does not give rise to any concerns about allergy in humans. Furthermore, the yeast cream contains no proteins or peptides or other constituents that could potentially induce allergy. For the worker handling the yeast creams, exposure is minimal as the yeast cream is handled in closed systems. Being liquid, dust formation and allergenicity is not a concern.

6.7 Assessment of allergenicity of newly expressed protein

As explained above in section 6.2., it is extremely unlikely that the peptide arising from the one unidentified open reading frame on the expression plasmid would ever reach the extra-cellular environment of the viable *S. cerevisiae* cell. If it by any chance were to reach the extracellular environment from a dead lysed cell, then it would be denaturated by the heating process performed on all yeast cream. It is therefore concluded that there is no concern related to allergenicity of the potential newly expressed protein.

6.8 Assessment of allergenicity of the whole GM product

Just as for the QPS yeast cream as a feed material, NOVO Yeast Cream from the GM yeast strain as feed material does not give rise to any concerns about allergy in humans. The yeast cream contains no proteins or peptides or other constituents that could potentially induce allergy. Furthermore, for the worker handling the yeast cream, exposure is minimal as the yeast cream is handled in closed systems. Being liquid, dust formation and allergenicity is not a concern. Allergenicity in target animals is not a concern.

6.9 Nutritional assessment

6.9.1 Nutritional assessment of GM food

Not relevant for a feed material

6.9.2 Nutritional assessment of GM feed

NOVO Yeast Cream has been nutritionally assessed a number of times. It is an excellent source of high quality protein for the pig market that fits well into the traditional wet-feeding systems. The high content of salts does not have a negative impact on performance of the pigs.

A recent study investigated the protein and energy digestibility of NOVO Yeast Cream in a balance study with Wistar rats. The spray-dried yeast cream was compared with soya bean meal. The results are very briefly listed in the table below.

Digestibility and protein utilization of the total diet (experimental feed + N-free basal mixture)		
	Soya bean meal	**Yeast cream**
Dry matter, %	89.8	89.4
Digestibility, %		
Protein (apparent)	83.8	85.9
Protein (true)	86.9	88.8
Protein utilization		
Retained N, % of N intake	41.7	46.3
Retained N, % of dig. N	50.1	46.0

A comparison of NOVO Yeast Cream's nutritional content with that of three conventional counterparts (listed above in Section C.5) shows that the NOVO product is nutritionally very close to the conventional yeast biomass feed materials.

Tentative pig production results from 2008 that compare pig herds fed NOVO Yeast Cream with other pig herds imply that the NOVO Yeast Cream pigs perform equally as well as the conventionally fed pigs.

NOVO Yeast Cream has high concentrations of phosphorous, sodium, potassium, and chloride. Especially the high content of chloride increases the animal's uptake of water. Therefore, the farmers are recommended to ensure that the animals always have free access to water. The high content of chloride limits the use of yeast cream in the feed to about 20-40% of wet weight feed.

6.10 Post-market monitoring of GM products

As NOVO Yeast Cream does not contain any viable GMO's or any transferable DNA, we find that post-market monitoring is not necessary.

It can be noted that there have never been post-marketing findings of any sort for pigs fed NOVO Yeast Cream since its start as a feed material in 1989. The NOVO Yeast Cream is currently only marketed in Denmark, and every pig sent to slaughter in Denmark is examined ante-mortem as well as post-mortem. The ante-mortem procedure comprises a thorough clinical examination upon arrival to the abattoir. Any clinical symptoms are examined in detail. The post-mortem examination comprises a macroscopic examination for any pathological lesions in every single animal. Samples are taken for bacteriological examination if indicated by macroscopic findings. All pigs are given a unique number so any finding can be traced back to the farm. Nothing has ever been report that can be linked to the NOVO Yeast Cream.

D. POTENTIAL ENVIRONMENTAL IMPACT OF GMMS AND DERIVED PRODUCTS

1. Environmental Assessment for Level 1 cases

1.1 Spread of the GMM from the product to external environments

The GM strain of *Saccharomyces cerevisiae* in the feed material in this application is heat-killed before being placed on the market. Therefore, it cannot spread to the external environment as living organisms and is likewise not metabolically active.

1.2. General ability of the GMM to survive and persist in external environments

The GM strain of *S. cerevisiae* is heat-killed before it reaches the external environment so neither survival nor persistence in the external environment is relevant. Data to support its inability to persist as a living organism in the external environment have been generated by three field investigations between 1993 and 2002, all conducted in the vicinity of the production facility in Kalundborg, Denmark. In none of the investigations could the GM *S. cerevisiae* be found outside of the production facility.

1.3 Transfer of recombinant DNA

The rDNA of the yeast cannot be transferred to other organisms. First of all, the GMM yeast in the yeast cream is dead, so transfer of DNA that would be driven by the living yeast cell is not possible. Secondly, the yeast's DNA is highly fragmented and, indeed, largely degraded; the probability of unit length reading frames of rDNA still existing in NOVO Yeast Cream is therefore virtually negligible. During its production, the yeast cream is heat treated at a high temperature. The dossier presents experimental data to support the fragmentation and degradation of the rDNA and gives additional explanation of what happens to DNA during such heat treatment. Finally, the growth of the silage culture also contributes to degradation of the rDNA.

2. Environmental assessment for Level 2 cases

The dossier only relates to Level 1, as described above.

3. Environmental monitoring plan

3.1 General

NOVO Yeast Cream is a residue after the commercial manufacture of an insulin precursor. This peptide is produced by the fermentation of a genetically modified strain of yeast, whose lineage from QPS yeast is clear and well-characterized. This production process is very carefully controlled and closely monitored. After extraction of the medicinal product, the residual yeast biomass is heat treated to secure total kill of the microorganism and fragmentation of the DNA. That is done in a verified process and ensures that there are no live GMO's in NOVO Yeast Cream. In Denmark each individual pig that is slaughtered is identifiable and traceable, which would ensure the tracing back of any adverse effect seen in a pig which had been fed the yeast cream.

3.2. Interplay between environmental risk assessment and monitoring

3.2.1 Monitoring of effects: foreseen and unforeseen

The formal environmental risk assessment did not foresee any adverse effects on the environment of the GM *S. cerevisiae* or any GM-specific adverse effects resulting from the production. There is surveillance of every individual pig slaughtered in Denmark, which ensures the tracing of any observed adverse effect in the animal.

3.2.2 Monitoring framework

Not applicable.

3.3 Case-specific GM monitoring

Not relevant, as there are no live GMO's in the NOVO Yeast Cream.

3.4 General surveillance of the impact of the GMM

3.4.1 Approach and principles

Novo Nordisk monitors its production by the GMM yeast very closely. In addition, every pig slaughtered in Denmark is subjected to careful examination both ante- and post-mortem, and each pig is given a unique identifier allowing the tracing of any adverse effects back to the farm.

3.4.2 Main elements of General Surveillance

See D.3.1. above.

3.5 Monitoring systems

The sections above give an overview of the monitoring systems that are enlisted for general surveillance of slaughtered pigs which have been fed Novo Yeast Cream.

3.6 Reporting the results of monitoring

As part of the Danish reporting system for animals for food, all findings are unique in the sense that each pig has been given a unique identification number. All pathological findings during ante-mortem and/or post-mortem inspection are collated in a database maintained by the Danish Veterinary and Food Administration (http://www.uk.foedevarestyrelsen.dk/Forside.htm)

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

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

a) Notification number Not relevant. The Danish competent authorities have officially acknowledged Novo Nordisk's use of GM yeast cream as pig feed since 1988, although official approval under the conditions of use by Novo Nordisk was not required by Danish law, as explained in the letters of acknowledgement. Novo Nordisk is registered and approved as a feed business operator in accordance with Regulation 183/2005 on feed hygiene. The Danish competent authority for feed, the Plant Directorate, has given Novo Nordisk the number 208-R890138 in the authority's database of feed business operators.
b) Conclusions of post-release monitoring Every pig slaughtered in Denmark is traceable by a unique identifier. In the 21 years NOVO Yeast Cream has been used as feed for the pigs, no adverse effects have ever been reported in connection with use of it as feed.
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 Directive 2001/18/EC) At intervals since 1988, the Danish Competent Authority has received information from Novo Nordisk on the use of NOVO Yeast Cream as pig feed. No risk to human health or the environment has ever become apparent.

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

a) Duration of post-releases monitoring
b) Conclusions of post-release monitoring
c) Results of the release in respect to any risk to human health and the environment
d) Release country No releases have been made of the GMMs outside of the Community.
e) Authority overseeing the release
f) Release site
g) Aim of the release

h)	Duration of the release
i)	Aim pof post-releases monitoring

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
b)	Assessment Report of the Competent Authority (Directive 2001/18/EC)
c)	EFSA opinion
d)	Commission Register (Commission Decision 2004/204/EC[1] http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=19
e)	Molecular Register of the Community Reference Laboratory/Joint Research Centre http://gmo-crl.jrc.ec.europa.eu/summaries/pMT742method-SANCO.pdf
f)	Biosafety Clearing-House (Council Decision 2002/628/EC)[2]
g)	Summary Notification Information Format (SNIF) (Council Decision 2003/812/EC)

[1] *Commission Decision of 23 February 2004 laying down detailed arrangements for the operation of the registers for recording information on genetic meodifications in GMOs, provided for in Directive 2001/18/EC of the European Parliament and of the Council. Official Journal of the European Communities L 6 5:20-22.*

[2] *Council Decision of 25 June 2002 concerning the conclusion, on behalf of the European Community, of the Cartagena Protocol on Biosafety. Official Journal of the European Communities L 201: 48-49.*