PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	Spain
(b)	Notification number	B/ES/06/40
(c)	Date of acknowledgement of notification	02/03/2006
(d)	Title of the project	**Safety in pregnant does and duration of immunity assessment of a vaccine based on the virus strain 6918VP60.**
(e)	Proposed period of release	From 17/04/2006 until 18/04/2007

2. Notifier

Name of institution or company: Laboratorios Syva, S.A. León (Spain)

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)

specify phylum, class …

(b) Identity of the GMO (genus and species)
Recipient organism: myxoma virus: *Poxviridae Chordopoxvirinae Leporipoxvirus*
Name of the GMO strain: 6918VP60.

(c) Genetic stability – according to Annex IIIa, II, A(10)

Strain 6918VP60 genetic stability has been investigated by serial passages in rabbits and also in RK13 cell line. In both cases there has been no virulence reversion of the strain. 6918 parental strain has also not been detected .

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (.) No (X)

If yes, insert the country code(s) …

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes (X) No (.)

If yes:

- Member State of notification Spain
- Notification number B/ES/05/26

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes (.) No (X)

If yes:

- Member State of notification ..
- Notification number ../../../…

7. Summary of the potential environmental impact of the release of the GMOs.
None.
Release will be done as a vaccination of wild rabbits kept in individual cages. The cages are situated in a small farm specially built for this trial. Rabbits have no contact with the environment.
No effects are expected.

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:
(select one only)

viroid (.)
RNA virus (.)
DNA virus (X)
bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)

- other animal (.)
  (specify phylum, class) …

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	…
(ii)	genus	*Leporipoxvirus*
(iii)	species	…
(iv)	subspecies	…
(v)	strain	6918
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	Myxomatosis virus

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (X) No (.) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (.)

If yes, indicate the type of ecosystem in which it is found:

Atlantic	..
Mediteranean	..
Boreal	..
Alpine	..
Continental	..
Macaronesian	..

(ii) No (.)
(iii) Not known (X)

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (X)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (X)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify	only rabbits

(b) If the organism is an animal: natural habitat or usual agroecosystem:
…

5. (a) Detection techniques
Isolation and identification on cell culture.
PCR

(b) Identification techniques
PCR

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (.) No (X)
If yes, specify
…

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (X) Not known (.)

If yes:

(a) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other (.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

Virus strain level P1.
Only infective for rabbits, very low virulence.
Not hazardous to human beings.

8. Information concerning reproduction
Not applicable

(a) Generation time in natural ecosystems:

(b) Generation time in the ecosystem where the release will take place:
…
(c) Way of reproduction: Sexual .. Asexual ..

(c) Factors affecting reproduction:
…

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	none known

(b) relevant factors affecting survivability:
Presence of rabbits, only susceptible species.

10. (a) Ways of dissemination
From rabbit to rabbit by direct contact and mecanically by arthropod vectors (fleas, mosquitoes).

(b) Factors affecting dissemination
Presence of arthropod vectors.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers).
The GMO has already been released previously, notification numbers B/ES/99/12 and B/ES/99/13 by other notifier.
The same notifier will release the same GMO as indicated on notification number B/ES/05/26 previously mentioned.

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i)	insertion of genetic material	(X)
(ii)	deletion of genetic material	(.)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify	…

2. Intended outcome of the genetic modification
Expression of heterologous protein VP60 and heterologous epitope DA3.

3. (a) Has a vector been used in the process of modification?
Yes (X) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?

Yes (X) No (.)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

plasmid	(X)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	…

(b) Identity of the vector
pUC19 plasmid with a replication origin from pBR322 plasmid.

(c) Host range of the vector
E.coli.

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes (X) No (.)

antibiotic resistance	(.)
other, specify	VP60 protein and DA3 epitope coding sequences

Indication of which antibiotic resistance gene is inserted
bla : ampicillin resistence (Ap^R)

(e) Constituent fragments of the vector

pUC19 plasmid

	fragment	**origin**
1- 137	2074-2210	pBR322
138- 237	2252-2351	pBR322
238- 395	1461-1304 (c)	lac operon
396- 452	57- 1	polylinker from vector M13mp19
455- 682	1298-1071 (c)	lac operon
683-2686	2352-4355	pBR322

(f) Method for introducing the vector into the recipient organism

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)

(iv) microinjection (.)
(v) infection (.)
(vi) other, specify Homologous recombination

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i) transformation (.)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify …

6. Composition of the insert

(a) Composition of the insert

1.-Early/late sintetic poxvirus promoter

2.-VP60 sequence

3.-DA3 epitope sequence

4.- Sequences from transference plasmids .

(b) Source of each constituent part of the insert
See (a).

(c) Intended function of each constituent part of the insert in the GMO

1.-Early/late sintetic poxvirus promoter: gene expression promoter

2.-VP60 sequence: immune response

3.-DA3 epitope sequence: immune response

4.-Sequences from transference plasmids : none expected, are just residues of the transference plasmid.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (X)
- other, specify …

(e) Does the insert contain parts whose product or function are not known?
Yes (X) No (.)
If yes, specify …
Sequences derived from transference plasmids

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

viroid		(.)
RNA virus		(X)
DNA virus		(.)
bacterium		(.)
fungus		(.)
animal		
-	mammals	(.)
-	insect	(.)
-	fish	(.)
-	other animal	(.)
	(specify phylum, class)	…
other, specify		…

2. Complete name

(i)	order and/or higher taxon (for animals)	
(ii)	family name	*Caliciviridae*
(iii)	genus	*Lagovirus*
(iv)	species	…
(v)	subspecies	…
(vi)	strain	AST89
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	*Rabbit Hemorrhagic Disease Virus (RHDV)*

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (X) No (.) Not known (.)

If yes, specify the following:

(a) to which of the following organisms:

humans	(.)
animals	(X) (only rabbits)
plants	(.)
other	..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

Yes (.) No (X) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):

…

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (.) No (X)

If yes, specify …

5. Do the donor and recipient organism exchange genetic material naturally?

Yes (.) No (X) Not known (.)

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?

Yes (.) No (X) Not known (.)

Specify Possibilities for the GMO to survive better than the parental strain are supposed to be nearly none. The genetic modification has involved only structural genes and there is no modification of original genes of the parental mixoma strain as the information has been inserted in a intergenic position..

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (.) No (X) Unknown (.)

Specify as far as for the studies of multiplicity on cell cultures is concerned both strains behave in a similar way.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes (.) No (X) Not known (.)

Specify following the studies of selection of the recipient strain and the studies of safety of GMO in rabbits.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes (.) No (X) Not known (.)

Specify See point (c).

2. Genetic stability of the genetically modified organism

Strain 6918VP60 genetic stability has been investigated by serial passages in rabbits and also in RK13 cell line. In both cases there has been no virulence reversion of the strain. 6918 parental strain has also not been detected.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes (.) No (X) Unknown (.)

(a) to which of the following organisms?

humans	(.)
animals	(.)
plants	(.)
other	…

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)
…

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Specific PCR able to detect mixoma virus and to distinguish between parental strain and GMO.

(b) Techniques used to identify the GMO
Specific PCR able to detect mixoma virus and to distinguish between parental strain and GMO.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
To study:
- safety of the vaccine based on the strain 6918VP60 in pregnant does
- duration of immunity in rabbits vaccinated with the vaccine based on the strain 6918VP60

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?
Yes (X) No (.)
If yes, specify …

Trials will not be done in free ranging animals. Rabbits will be kept in individual cages.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):

Location

Cinegética Monteagudo
Valbón 12-B
36190 Agudelo (San Martiño)
Barro
Province of Pontevedra
Galicia (Spain)

UTM

X: 529063

Y: 4708251

(c) Size of the site (m^2):

Building of the trial : 68 m^2

Location in wich the building is: : 1800 m^2

(i) actual release site (m^2): 68 m^2

(ii) wider release site (m^2): 1800 m^2

(d) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
No.

(e) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
None, rabbits are in cages.

4. Method and amount of release

(a) Quantities of GMOs to be released:

- Safety in pregnant does : 3 x 10^5 PFU
- Duration of immunity: 1 x 10^5 PFU

(b) Duration of the operation:

- Safety in pregnant does : 68 days.
- Duration of immunity: 1 year.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release

- Rabbits will be in individual cages. Arthropod vectors will be absent.

5. Short description of average environmental conditions (weather, temperature, etc.)
Weather is temperate rainy climate without extremes of temperature and precipitation.

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.
The same GMO has been already authorized for release three times.
Authorization numbers are B/ES/99/12 and B/ES/99/13 for the already done releases, with is no information on health impacts.
There is another release (B/ES/05/26) that has not been done yet.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

No interaction with the environment is expected because rabbits are captive in individual cages and will have no contact with other individuals.

1. Name of target organism (if applicable)

(i) order and/or higher taxon (for animals) …
(ii) family name for plants …
(iii) genus …
(iv) species …
(v) subspecies …
(vi) strain …
(vii) cultivar/breeding line …
(viii) pathovar …
(ix) common name …

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
Not applicable.

3. Any other potentially significant interactions with other organisms in the environment
Not applicable.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (X) Not known (.)
Give details
The selection would only occur in rabbits that are captive and isolated from environment.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
None.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO
None.
(i) order and/or higher taxon (for animals) …
(ii) family name for plants …
(iii) genus …
(iv) species …
(v) subspecies …
(vi) strain …
(vii) cultivar/breeding line …
(viii) pathovar …
(ix) common name …

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
None, there is contact between captive and free ranging rabbits.

(b) from other organisms to the GMO:
None, same reason.

(c) likely consequences of gene transfer:

None.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):

   There is no such a study available.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
   Not applicable.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
   This GMO has no opportunity to stablish nor disseminate because there no contact between the vaccinated rabbits and free-living wild rabbits.
   To detect the GMO, most specific and sensitive techniques are molecular ones, mainly PCR , with a sensitivity of 0,2 PFU.
   The trials involve daily monitoring of rabbits during the study,clinical examinations and serological tests.

2. Methods for monitoring ecosystem effects
   There are no ecosystem effects expected, all the monitoring will be done on captive rabbits.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
   PCR techniques are specific to detect specific genes (VP60 and DA3), but there is no transfer possibility as there are no free living hosts.

4. Size of the monitoring area (m^2)
   68 m^2 .

5. Duration of the monitoring
   - Safety in pregnant does : 68 days
   - Duration of immunity: 1 year.

6. Frequency of the monitoring
   Daily .

I. Information on post-release and waste treatment

1. Post-release treatment of the site
   Disinfection by means of hypochlorite after organic matter removal.

2. Post-release treatment of the GMOs
   Vaccine used vials and rabbit carcasses will be decontaminated by autoclaving.
   Rabbit faeces will be processed by authorized company named GESUGA.
   Tools, etc. will be disinfected by hypochlorite containing solutions.
   The building will be dismantled after trial.

3. (a) Type and amount of waste generated
A maximum of 1000 l of residues in total.

(b) Treatment of waste
Heat inactivation (autoclaving or cremation).

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
The spread of the GMO should be only if there are contact between captive and free ranging rabbits; then the first step should be the disposal of rabbits and arthropod vectors.

2. Methods for removal of the GMO(s) of the areas potentially affected
Disposal of the rabbits and arthropod vectors. Safe disposal of carcasses.
Deep cleaning and disinfection of tools, yards and other objects.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
See 2.

4. Plans for protecting human health and the environment in the event of an undesirable effect
There is no human health risk with myxomatosis viruses.
The myxomatosis virus does not affect the environment.