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	Italy
(b)	Notification number	B/IT/10/01
(c)	Date of acknowledgement of notification	09/06/2010
(d)	Title of the project	"Improve tools and strategies for the prevention and the control of classical swine fever"
(e)	Proposed period of release	From 01/10/2010 until 31/10/2010

2. Notifier

Name of institution or company: Istituto Zooprofilatico Sperimentale Umbria Marche

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class …

(b) Identity of the GMO (genus and species)
Genus: Pestivirus
Species: Bovine Viral Diarrhea Virus

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

Full genomic sequence analysis and genetic stability analysis of the GMO were done. Table 1 shows the sequence analysis of the 11th passage of virus obtained after virus rescue post transfection of SK-6 cells with the cDNA construct pA/CP7_E2alf. The nucleotide sequence comparison between the 11th passage of CP7_E2alf and the GMP-produced stock virus generated for the animal experiments led to the identification of 10 mutations. These mutations result in nine amino acid substitutions and one insertion of a nucleotide located in the 5′ untranslated region (UTR) (Table 1). Only one nucleotide exchange in the E2 protein located at its C-terminus was identified. This exchange leads to an amino acid substitution from serine to proline but does not affect known B- or T-cell epitopes in this region. The other nucleotide exchanges concern the BVDV backbone and do not influence the induction of CSFV E2 specific neutralizing antibodies.
Table 1.

Tab. 1 - Sequence differences between CP7_E2alf of the 11th passage after transfection and the CP7_E2alf virus stock produced under 'good manufacturing conditions' (GMP; Fort Dodge, Spain). The numbering is according to the BVDV CP7 sequence (accession no. U63479). Nucleotide exchanges and the resulting amino acid shifts are listed. The corresponding viral proteins are shown.

Nucleotide position	**Nucleotide exchange**	**Amino acid substitution**	**UTR or protein coding sequence**
78	Insertion of A	–	5′UTR
330	C → T	–	5′UTR
1068	G → A	D → N	Capsid
1800	C → T	L → F	E^{rns}
3309	T → C	S → P	E2-Alfort
3580	A → G	Y → C	p7
5049	G → T	V → L	NS2
7737	A → G	M → V	NS4B
10393	G → T	S → I	NS5B
12022	C → A	T → K	NS5B

Reference: Leifer I., Lange E., Reimann I., Blome S., Juanola S., Plana Duran J., Beer M., 2009. Modified live vaccine candidate CP7_E2alf provides early onset of protection against lethal challenge infection with classical swine fever virus after both intramuscular and oral immunization. Vaccine, 27, 6522-6529

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 (.) No (X)

If yes:

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

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 B/../../…

7. Summary of the potential environmental impact of the release of the GMOs.

The potential environmental impact of the GMO is valuable as minor or absent. It can be exclude that GMO is shed from the primary recipient. Theoretically, but never observed in practice, the GMO could not recombine with wild type pestivirus in a recipient infected with both viruses. Animal experiments do not suggest that the tropism modification changes its biodistribution.
Further horizontal transmission of the GMO via respiratory tract infections is expected to be different from that of wild type pestivirus, because it is not able to evidence symptoms and it is selflimiting. Also the infection by the GMO of secondary recipient (such as wild ruminants) infections is not expected to be any different from that of primary one.
After oral vaccination with either different CP7_E2alf dilutions or C-strain vaccine, no clinical reactions were observed and all groups of wild boars showed normal body temperature.
Animals vaccinated with undiluted CP7_E2alf or CP7_E2alf diluted 1:10 did not show any clinical symptoms upon challenge infection. In addition, body temperature stayed within the physiological range although a slight increase was observed between days 3 and 5 in most animals.

Reference: Leifer I., Lange E., Reimann I., Blome S., Juanola S., Plana Duran J., Beer M., 2009. Modified live vaccine candidate CP7_E2alf provides early onset of protection against lethal challenge infection with classical swine fever virus after both intramuscular and oral immunization. Vaccine, 27, 6522-6529

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

1. Recipient or parental organism characterization:

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

(select one only)

viroid	(.)
RNA virus	(X)
DNA virus	(.)
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	Pestivirus
(iii)	species	Bovine Viral Diarrhea Virus
(iv)	subspecies	…
(v)	strain	BVDV/CSFV chimera CP7_E2alf
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	…

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 (X)

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

Atlantic	X
Mediteranean	X
Boreal	X
Alpine	X
Continental	X
Macaronesian	x

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

(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	The vaccine has not a natural habitat: this vaccine could be considered only in case of emergency vaccination to control the CSF spread in wild boar population. Only the European Commission can authorize this emergency vaccination campaign against CSF

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

5. (a) Detection techniques
As the wild type pestivirus, also the BVDV/CSFV chimera CP7_E2alf is detectable by cell culture and/or (quantitative) PCR.

(b) Identification techniques
As the wild type pestivirus, also the BVDV/CSFV chimera CP7_E2alf is identifiable by sequencing, restriction digest analysis and PCR. Vaccinated animals could be indentified by serum ELISA technique: this method permits the differentiation between vaccinated and infected animals

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (X) No (.)
If yes, specify
- Council Directive 2001/89/EC of 23 October 2001 on Community measures for the control of classical swine fever
- Commission Decision 2002/106/EC of 1 February 2002 approving a Diagnostic Manual establishing diagnostic procedures, sampling methods and criteria for evaluation of the laboratory tests for the confirmation of classical swine fever

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

Infectious cDNA clones of CSFV and BVDV permit the construction of deletion mutants, chimeric viruses, and replicons. Chimeric pestivirus based on the infectious DNA copy of BVDV strain CP7 was constructed replacing the BVDV E2 protein by the E2 protein from CSFV strain Alfort 187 (CP7_E2alf). Following intramuscular inoculation, CP7_E2alf proved to be completely avirulent. Neither viremia nor virus transmission to contact animals could be detected. CSFV-specific neutralising antibodies were detected from day 11 post-inoculation onwards. All animals scored positive in an E2-specific CSFV-antibody ELISA but negative for CSFV-E^{RNS}-specific antibodies in a CSF marker ELISA. After challenge infection with highly virulent CSFV, all pigs immunised with CP7_E2alf were fully protected against clinical signs, viremia and virus shedding. Twenty one days post challenge all pigs scored positive in a CSFV marker ELISA. Therefore pigs immunised with these chimeras were completely protected against lethal CSFV infection, and no virus transmission to contact animals was observed.

Reference: Beer M., Reimann I., Hoffmann B., Depner K., 2007. Novel marker vaccines against classical swine fever. Vaccine, 25, 5665-5670

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

A chimeric BVDV/CSFV full-length clone was constructed, in which the entire E2-coding region of cytopathogenic BVDV strain CP7 was replaced by that of CSFV strain Alfort 187. Concerning replication and cell tropism, the generated chimeric *Pestiviruses* reported so far displayed varying phenotypes in vitro. The CSFV/BVDV chimera expressing the N-terminal portion of BVDV II-derived E2 in a CSFV background grew to virus titers, which were reduced not more than 10-fold compared to wild-type CSFV in porcine kidney cells. It was therefore concluded that the replacement of the antigenic region of E2 did not change cellular tropism of C-strain-based CSF viruses for porcine SK6 and bovine FBE cells.

Reference: Reimann I., Depner K., Trapp S., Beer M., 2004. An avirulent chimeric Pestivirus with altered tropism protects pigs against lethal infection with classical swine feve virus. Virology, 322, 143-157

(b) Generation time in the ecosystem where the release will take place:

the GMO replicates with a generation time similar to that of wild type, but the replication is limited only to the primary target organs of vaccinated animals

(c) Way of reproduction: Sexual .. Asexual X

(c) Factors affecting reproduction:

Theoretically there are no differentiation between the GMO and the wild type

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 …	

(b) relevant factors affecting survivability:

At ambient temperature and high relative humidity pestiviruses may survive for several days on various surfaces. In frozen condition, the virus can be kept for years.

10. (a) Ways of dissemination

Dissemination may occur by ingestion of oral bates.

(b) Factors affecting dissemination

Climate factors can influencing the survival of GMO in the bates. High temperature, direct sun exposition as well as high humidity level can reduce the survival of the vaccine

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)

Not applicable

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

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

Wild boar are an important reservoir of Classical swine fever virus (CSFV) in several European countries, where most of the primary outbreaks in domestic pigs are directly related to the endemic disease situation in the wild boar population. Oral immunisation has been introduced as an additional control measure to accelerate CSF eradication in wild boar in Germany since 1993. Immunisation with an oral bait vaccine based on the conventionally attenuated live vaccine strain “C” proved to be safe and effective, but does not allow differentiation between infected and vaccinated animals. Therefore, we examined the vaccine efficacy of the recently constructed chimeric pestivirus CP7_E2alf, whose coding sequences for the major envelope protein E2 of BVDV strain CP7 are replaced by E2 of the CSFV strain Alfort187. Following oral immunisation of wild boar, CP7_E2alf proved to be completely avirulent. Furthermore, all vaccinees were fully protected from clinical disease after a highly virulent CSFV challenge infection. The immunised animals seroconverted within 3 weeks after vaccination for CSFV E2-specific and CSFV neutralising antibodies, whereas prior to challenge infection no antibodies against CSFV E^{rns} were detected with an

appropriate CSFV-specific marker ELISA test. Thus, the BVDV backbone of CP7_E2alf enables serological and genetic differentiation from wild type CSFV infection. In conclusion, CP7_E2alf represents the first efficient and safe marker vaccine candidate for oral immunisation of wild boar against CSFV.

Reference: Reimann I, Depner K, Trapp S, Beer M. An avirulent chimeric pestivirus with altered cell tropism protects pigs against lethal infection with classical swine fever virus. Virology 2004;322(1):143–57]

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

pA/BVDV; pA/CP7_ΔE2PacI

(c) Host range of the vector

In vitro transcription of linearized full-length cDNA constructs was performed by T7 RiboMax Large Scale RNA Production System (Promega) according to the manufacturer's instructions. The amount of RNA was estimated by ethidium bromide staining following agarose gel electrophoresis. For transfections, 1×10^7 PK15 or KOP-R cells were detached using a trypsin solution, washed with PBS, mixed with 1–5 µg of in vitro-synthesized RNA and transfected (two pulses at 850 V, 25 µF, 156 Ω) using an *EasyjecT Plus* (EquiBio) electroporation unit.
For virus recovery, in vitro-transcribed RNA (1–5 µg) was transfected into PK15 or KOP-R cells by electroporation. Cell culture supernatants or complete cell lysates after freezing/thawing were harvested at 24 h to 120 h post-transfection (p. t.), and titrated using KOP-R or PK15 cells.

Reference: Reimann I., Depner K., Trapp S., Beer M., 2004. An avirulent chimeric Pestivirus with altered tropism protects pigs against lethal infection with classical swine feve virus. Virology, 322, 143-157

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

Yes (.) No (X)

antibiotic resistance (.) not applicable
other, specify not applicable

Indication of which antibiotic resistance gene is inserted
not applicable

(e) Constituent fragments of the vector
…

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

(i) transformation (.)
(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specify … transfection

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
envelope protein E2-encoding region of classical swine fever virus (CSFV) strain Alfort 187

(b) Source of each constituent part of the insert
…

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

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify plasmid pA/CP7_E2alf

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

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 for plants	…
(iii)	genus	Pestivirus
(iv)	species	Classical Swine Fever Virus
(v)	subspecies	…
(vi)	strain	CSFV strain Alfort 187
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

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:

(c) to which of the following organisms:

humans	(.)
animals	(X)
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 …

(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 …

(d) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (X) Not known (.)
Specify …

(e) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (X) Not known (.)
Specify …

2. Genetic stability of the genetically modified organism
The GMO is stable. genetic stability was tested after multiple passaging in cell culture.

Reference: Reimann I., Depner K., Trapp S., Beer M., 2004. An avirulent chimeric Pestivirus with altered tropism protects pigs against lethal infection with classical swine feve virus. Virology, 322, 143-157

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) Detection techniques
As the wild type pestivirus, also the BVDV/CSFV chimera CP7_E2alf is detectable by cell culture and/or (quantitative) PCR.

(b) Identification techniques
As the wild type pestivirus, also the BVDV/CSFV chimera CP7_E2alf is identifiable by sequencing, restriction digest analysis and PCR.
Vaccinated animals could be indentified by serum ELISA technique: this method permits the differentiation between vaccinated and infected animals

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
Due to the vast economic consequences of classical swine fever (CSF) outbreaks, emergency vaccination plans are under discussion in European Union Member States. However, animals vaccinated with the conventional C-strain vaccine are subject to trade restrictions. To ease these restrictions, potent marker vaccines are required. One promising candidate is the chimeric pestivirus CP7_E2alf. For emergency vaccination in a CSF outbreak scenario, early onset of immunity is required. Here, the studies performed with a CP7_E2alf virus stock produced under good manufacturing conditions (GMP) are reported. In challenge experiments, CP7_E2alf induced full clinical protection 1 week after intramuscular vaccination, and 2 weeks after oral immunization. Furthermore, even after application of diluted vaccine preparations complete protection could be achieved if challenge infection was carried out 4 weeks after vaccination. In conclusion, GMP-produced CP7_E2alf proved to be a suitable marker vaccine candidate – also for emergency vaccination – both after intramuscular and oral application.

Reference: Leifer I., Lange E., Reimann I., Blome S., Juanola S., Plana Duran J., Beer M., 2009. Modified live vaccine candidate CP7_E2alf provides early onset of protection against lethal challenge infection with classical swine fever virus after both intramuscular and oral immunization. Vaccine, 27, 6522-6529

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 (.) No (.)

If yes, specify …

3. Information concerning the release and the surrounding area

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

Farm A: Name Roscetti - national register code 013 PG 136 - Surface 577 ha – Municipality Città di Castello, Umbria Region Italy

Farm B: Name Schifanoia –national register code 057 PG 024 - Surface 52499 ha – Municipality Gubbio, Umbria Region Italy

Farm C: Name Paradiso di Pianciano - national register code 051 PG 024 - Surface 1187 ha – Municipality Spoleto, Umbria Region Italy

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not done

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Wild ruminant species

4. Method and amount of release

(a) Quantities of GMOs to be released:
15/20 bates /1ha

(b) Duration of the operation:
1 month

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
The bates for oral vaccination will be posed in areas where wild boars are habitually foddered

5. Short description of average environmental conditions (weather, temperature, etc.)
The vaccination will be performed in autumn: in this period The climate of Umbria Region is temperate.

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.
Not applicable

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

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	Suide
(ii)	family name for plants	…
(iii)	genus	Sus

(iv)	species	scrofa
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Wild Boar

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
the wild boar, after the ingestion of bates containing the GMO, is expected to elicit an immune response against the antigen.

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
…

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
…

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

It is not excludible the ingestion of bates by wild animals, but no significantly harmed consequences are expectable for non target organism.

(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:
As described in other sessions of this document, the genetic stability of the GMO was tested and moreover the survival of the GMO after the releasing is limited.

(b) from other organisms to the GMO:
Not applicable

(c) likely consequences of gene transfer:
Not applicable

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.):
Not applicable

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
Wild boar population object of vaccination campaign will be monitored: after the vaccination the wild boar population will be hunt in the selected areas until the elimination: this areas, in fact, are called 'faunistic-hunting farms' (FHF). The hunted wild boars will be controlled by pos mortem examination; in this occasion blood sample will be collected for direct (PCR test) and indirect (ELISA Test) investigation against chimeric pestivirus CP7_E2alf

2. Methods for monitoring ecosystem effects
Video surveillance will be implemented for monitoring of the areas where the vaccine bates will be released

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

Serological surveillance plans will be implemented in the animal population living in the 'faunistic-hunting farms' involved in the study

4. Size of the monitoring area (m^2)
Farm A: Name Roscetti - national register code 013 PG 136 - Surface 577 ha – Municipality Città di Castello, Umbria Region Italy
Farm B: Name Schifanoia –national register code 057 PG 024 - Surface 52499 ha – Municipality Gubbio, Umbria Region Italy
Farm C: Name Paradiso di Pianciano - national register code 051 PG 024 - Surface 1187 ha – Municipality Spoleto, Umbria Region Italy

5. Duration of the monitoring
1 year

6. Frequency of the monitoring
The frequency of monitoring depending on hunting bag

I. Information on post-release and waste treatment

1. Post-release treatment of the site

After 20 days the release of the bates the unheated ones will be removed

2. Post-release treatment of the GMOs
The removed bates will be sterilized

3. (a) Type and amount of waste generated
Not done

3. (b) Treatment of waste
Not done

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
The experimental pre-studies demonstrated that after vaccination the animals do not spread the virus

2. Methods for removal of the GMO(s) of the areas potentially affected
The vaccine bates will removed by hand: no risk for human are supposable

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
Not applicable

4. Plans for protecting human health and the environment in the event of an undesirable effect
Not applicable