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

- ENGLISH -

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 — Belgium
   (b) Notification number — B/BE/04/~~V1~~ BV1
   (c) Date of acknowledgement of notification — 27/Feb/03
   (d) Title of the project — Evaluation of the safety of a Feline Herpes virus, bivalent gene deleted live vaccine, administered as intranasal vaccination to cats
   (d) Proposed period of release — From .01/07/2004. until 01/07/2008

MG SBB 13.09.04

2. Notifier

Name of institution or company:

Pfizer Animal Health
Veterinary Medicine R&D, ipc 821
Ramsgate Road
Sandwich, Kent CT13 9NJ, UK

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(X.) The insert originates from a RNA virus
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)

specify phylum, class: Feline Herpes virus Type 1

(b) Identity of the GMO (genus and species)
Feline Herpes Virus Type 1

(c) Genetic stability – according to Annex IIIA, II, A(10)
The vaccine is comprised of two FHV vectors with a ~345 bp insertional deletion in the TK locus, conferring the TK-negative phenotype (?TK). The attenuated FHV vectors were generated by a process of homologous recombination, in which the flanking TK sequences around the FIV expression cassettes act as homologous sequence crossover sites with the wild-type FHV genome. To confirm the genetic stability, rFHV-FIVenv and rFHV-FIVgag constructs were passaged sequentially five times in cats. Viruses recovered from oro-pharyngeal tissues after five passages and original inocula were analyzed by PCR to demonstrate genetic stability. The PCR data indicated that the PCR products generated from primers specific for the TK deletion and the FIVenv or FIVgag gene inserts confirm that the TK deletion and FIV gene inserts remained intact, properly located and stable as compared to the rFHV-FIVenv or rFHV-FIVgag material prior to passage in cats.

These data support the conclusion that the virulence-attenuating gene deletions in both vaccine components are genetically stable. For a detailed description of the Genetic Stability aspects of the GMO we refer to the Environmental Risk Assessment, paragraph 1.1.5.

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

Yes (X) No (.)

If yes, insert the country code(s) NL and IE

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 The Netherlands…
- Notification number B/../../…Not yet identified

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 (X) No (.)

If yes:

- Member State of notification USA Field trials will start mid 2004
- Notification number B/../../…Not applicable

7. Summary of the potential environmental impact of the release of the GMOs.
The information provided in the Enviromental Risk Assessment document indicates that the use of the vaccine will not negatively affect animal safety, public health, or the environment. The overall risk rating for the vaccine was determined to be low.

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

1.A Recipient or parental organism characterisation: All the information provided below under this paragraph 1A relates to the wildtype **Feline Herpes virus**.

(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)	Herpesviridae
(ii)	genus	…
(iii)	species	Alphaherpesvirinae
(iv)	subspecies	
(v)	strain	
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	Feline Herpes Virus

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (.) No (X) Not known (.)
Feline Herpes virus is prevalent worldwide and is ubiquitous in domestic cat populations. The virus can only infect feline species.

(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 (X)
(iii) Not known (.)

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

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

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	specific feline pathogen

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Not applicable

5. (a) Detection techniques

In cases of neonatal death, FHV can be recovered from liver, kidney and spleen. The whole abortus or placenta can be submitted for virus isolation. Although FHV infection causes abortion in pregnant cats, the virus is usually not recoverable from aborted material. The virus is detected in culture, some laboratories have found that herpesviruses are more easily recovered from rayon-dacron swabs than from wooden cotton-tipped swabs. Virus isolation can be attempted from nasal and conjunctival swabs. PCR can also be used to detect FHV.

(c) Identification techniques
See under 5(a)

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

According to the European Commission classification of infective microorganisms by risk group (Council Directive 90/679/EEC of 26 November 1990), Feline Herpes virus is classified in group 1, since this is a biological agent that is unlikely to cause human disease.

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

If yes:

(a) to which of the following organisms:

humans (.)

animals	(X) only to feline
plants	(.)
other	(.)

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

Clinical signs of FHV infection include fever, sneezing, bilateral conjunctival hyperemia, serous oculonasal exudates, and depression and inappetence. Most cats recover within 1 to 2 weeks. Permanent injury to the nasal respiratory epithelium and turbinates may result when young kittens are infected. Ocular manifestations include conjunctivitis and keratitis, with development of dendritic corneal ulcers. Abortion, stillbirths, and infertility have been observed in cats with herpesvirus infection. FHV may establish latent infection in cats, resulting in a persistent carrier state.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Replication of FHV Type 1 occurs after inhalation or oral ingestion in the epithelium of the upper respiratory tract, i.e. from the nostrils to the bifurcation of the trachea. Secondary virus spread and replication occurs in the epithelium of conjunctiva, lacrimal canals and throat area. Virus replication can also occur in the lower respiratory tract (bronchi, bronchioli). Viraemia is possible, but does not always occur. If viraemia is present, virus can spread to the osteoblasts in the skeleton, or can cause abortion due to spread through the placenta. The virus is mainly excreted in secretions of the nose, eyes and throat. Virus excretion lasts approximately 2 weeks, but the virus remains latent for life in 80% of the infected cats. The viral genome can then be demonstrated in the trigeminal ganglia. A latent infection can easily lead to active virus excretion by administration of corticosteroids or stress. Moving animals from one cage to another, parturition, lactation are all factors that can lead to active virus excretion of a cat with a latent FHV infection.

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

(c) Way of reproduction: Sexual .. Asexual ..
Not applicable

(c) Factors affecting reproduction:
Not applicable…

9. Survivability

(a) ability to form structures enhancing survival or dormancy: Not applicable

(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:
FHV may establish latent infection in cats, resulting in a persistent carrier state.

10. (a) Ways of dissemination
see 8(a)

(b) Factors affecting dissemination
see 8(a)

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 …	

The biological agent was made by inserting a casette encoding the FIV *env* and *gag* product into the thymidine kinase (tk deleted) locus of a FHV. The resulting FHV vector was attenuated due to a deletion in the tk locus into which the cassette encoding the FIV *env* and *gag* had been inserted.

2. Intended outcome of the genetic modification
The objective of the genetic modification was to obtain a non-virulent FHV virus expressing the two proteins of the FIV virus, that can be used as a vaccine in cats.

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	(.)
bacteriophage	(.)

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

(b) Identity of the vector
Feline Herpes Virus tk-

(c) Host range of the vector
Feline

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

antibiotic resistance (.)
other, specify Absence of thymidine kinase gene

Indication of which antibiotic resistance gene is inserted
No antibiotic genes were inserted

(e) Constituent fragments of the vector

Briefly, the sequences of known tk genes were compared and regions of high homology were used to design degenerate oligonucleotide primers. The polymerase chain reaction method of DNA amplification was then used to amplify a short region of the FHV tk gene. This amplified region was then used as a probe to clone the entire tk region from the FHV genome. Sequence analysis showed similarity with known herpesvirus tk sequences.

A bacterial plasmid was constructed in which the tk coding sequence was modified by deleting the nucleoside-binding domain of the deduced tk protein. This plasmid contains the entire FHV tk gene and flanking regions, but lacks tk coding sequences. A synthetic oligonucleotide polylinker was used to join these sites in the plasmid construction. This plasmid contains the deleted FHV tk gene and flanked regions. This plasmid was then modified as described below by inserting a transcription unit containing an early promoter, the FIV *env* or *gag* coding sequences, and polyadenylation sequences. Plasmids were co-transfected with an FHV wild type-DNA into a feline cell line, allowing for homologous recombination between the plasmid and viral DNA. Briefly, the tk-mutation was introduced into FHV by using calcium phosphate co-precipitation techniques to obtain homologous recombination between plasmid and herpesvirus genomic sequences. Recombinant progeny viruses were harvested and plaque purified.

FHV progeny viruses were harvested and plaque purified under conditions to select for recombinant tk- viruses. The resulting viruses were screened by restriction endonuclease analysis for the correct insertion of the FIV transcription unit and by Western blot for expression of the *env* or *gag* gene.

The resulting virus has a tk- phenotype and expresses the FIV *env* or *gag* gene product. The resulting recombinant virus was grown and expanded two time in feline cells and used for preparation of the Master Seed Virus stock.

The resulting recombinant viruses rFHVΔtkFIV*env* and rFHVΔtkFIV*gag* are then mixed in equal proportions.

…

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

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

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
see description under 4e

(b) Source of each constituent part of the insert

The source of the insert is the Feline Immunodeficiency virus (FIV). One feline herpes vector tk- contains the *env* gene from FIV and another feline herpes virus tk- vector contains the *gag* gene from FIV. Both constructs contain the CMV immediate early promoter, the FIV *gag* or *en*v coding sequences and bovine growth hormone polyadenylation sequences.

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

Promoter and polyadenalation sequences function as start and termination of transcription, respectively.

(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	(.)	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	Retroviridae
(iv)	species	Lentivirinae
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Feline Immunodeficiency virus

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:

(b) to which of the following organisms:

humans (.)
animals (X) Feline only
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 (.)
The FIV *gag* gene product encodes the precursor protein for the capsid proteins of FIV. In cats the FIV *gag* gene product (*gag* protein) elicits an immune response to FIV *gag*. There are no known tissue tropisms associated with the FIV *gag* gene.
The FIV *env* gene product is the envelope glycoprotein of FIV. It contains the major neutralizing epitopes and functions as a cellular attachment protein. In cats, the FIV *env* protein elicits an immune response to FIV *env*. There are no known tissue tropisms associated with the FIV *env* gene.

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 (X) No (.)

If yes, specify

According to the European Commission classification of infective microorganisms by risk group (Council Directive 90/679/EEC of 26 November 1990), Feline Immunodeficiency virus is classified in group 1, since this is a biological agent that is unlikely to cause human disease.

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: Assessments of the survivability of the vaccine strains and the wild type parent were studied using a non porous surface, kitty litter and water. The data demonstrated that both wild type FHV and the recombinant FHV-FIV constructs had similar rates of decrease in viability (see Environmental Risk Assessment report)

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

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

Specify : The deletion of the FHV TK alters the pathogenesis of the virus in the cat. The modification also reduces the amount of replication (duration and amplitude) in the primary target tissues. FHV vectors included in the vaccine have not demonstrated an altered tissue tropism that would be adverse to the host animal. To date, efforts to recrudesce latent rFHV-FIV*env* or rFHV-FIV*gag* viruses through the use of immunosuppressants have been unsuccessful.

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

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

Specify The tissue tropism of the vector vaccine strains has not been altered by the gene deletion and insertion compared to the wild type FHV strain.

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

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

Specify The modification of the Feline Herpes virus vector to a tk- phenotype will attenuate the organism to a non-virulent phenotype.

2. Genetic stability of the genetically modified organism

Studies were conducted in cats to measure the potential of the vaccine strains to revert to virulence. The method required the reversion to virulence of the vaccine strains through at least five serial passages in cats. Separate groups of 10-12 week old kittens were vaccinated intranasally with rFHV-FIV*env* or rFHV-FIV*gag* . Both vaccine components were recovered from all passages at a level that allowed intranasal inoculation of cats in subsequent passages. Both vaccine components replicated to similar titers in all subsequent passages. The stability of each vaccine component was confirmed via polymerase chain reaction (PCR) and Western blot analysis of the original virus inoculum and viruses recovered from the fifth backpassage. The interpretation of these data was that both strains of the vaccine were able to replicate in the oro-pharynx of the vaccinated cats. Further, the lack of clinical signs suggestive of FVR and unchanged replicative potential over five backpassages confirmed that neither strain was able to revert to a virulent phenotype. The stability of the attenuating phenotype (an insertional deletion in the TK locus of the FHV) and the inserted FIV open reading frame (ORF) were also confirmed by PCR and Western blotting of infected cells.

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

The GMO can be detected in the cat by swabbing the oro-nasal-conjunctival secretions.

Alpha herpesviruses are not particularly suited to a long term survival outside of the host. Detection of the GMO outside of the cat will therefore be difficult.

(b) Techniques used to identify the GMO

Techniques to identify the GMO is cell culture in combination with PCR.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

To collect safety data in cats after vaccination under field conditions. The product is intended as a vaccine for the vaccination of healthy cats in the prevention and /or reduction of FIV plasma burdens. Subsequent to reduced plasma burdens, this product aids in the reduction of clinical signs associated with FIV in vaccinated cats and in the reduction of FIV shed and

spread from vaccinated cats. Furthermore, this vaccine aids in the prevention of disease caused by FHV.

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 (X)
   If yes, specify ...

3. Information concerning the release and the surrounding area

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

   HG SBB (1) 13.09.04

   (b) Size of the site (m^2): ... m^2
   (i) actual release site (m^2): 20 m^2
   (ii) wider release site (m^2): ... m^2
   Cats will be used which arrive at a feline veterinary practice and which will go home with their owners after vaccination. The extent of the introductory location can therefore vary per veterinary practice.

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

   (d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
   Not applicable

4. Method and amount of release

   (a) Quantities of GMOs to be released:
   In total a minimum of 40 cats will receive the vaccine. The maximum amount will be 1000 cats. The targeted dose level will be between minimum and maximum release level for both strains of the vaccine. The maximum level is set at $10^{6.5}$ PFU per strain. The vaccine will be administered twice: at the age of 8-10 weeks and again at the age of 11-13 weeks. This is the prime and boost regime described for primary immunization of cats as detailed on the product label. Also older cats will be vaccinated.
   So if 40 cats are vaccinated the total maximum amount of GMO,s released are 40 x 2 x $10^{6.5}$ =8 x $10^{7.5}$ PFU. In the case of 1000 cats the total maximum amount will be 1000 x 2 x $10^{6.5}$ = 2 x $10^{9.5}$ PFU.

   (b) Duration of the operation:
   Cats will be vaccinated twice with a 3-4 week interval. After the second vaccination cats will be observed for another two weeks. All cats will not be vaccinated at one day but in a timescale of 1-4 months. So the total duration of the observation is approximately 5,5 months.

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

(1) correction by SBB after modification and complementary information received for the technical dossier

Immediately after the vaccination the environment will be disinfected with standard methods. No special precautions are taken thereafter.

5. Short description of average environmental conditions (weather, temperature, etc.)
The field trail starts in autumn and will continue through winter time. Weather and temperature conditions are average Belgium conditions during this period of the year.

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.
This GMO has only been released under contained use conditions. The impact on the environment and the human health aspect are described in chapter 2 and 3 of the Environmental Risk Assessment. The overall conclusion was that the data suggest with a high degree of certainty that there is a low likelihood of risk to human health from the use of the vaccine. Also with a moderate degree of certainty that there is a low likelihood of adverse events associated with the release of the vaccine into the environment.

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

Not applicable.

1. Name of target organism (if applicable) Not 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 (.) 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

Not 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	…

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:

The likelihood of a genetic exchange is described in paragraph 1.1.5 of the Environmental Risk Assessment. The overall conclusion of the calculated risk of such an event was extremely low.

(b) from other organisms to the GMO:

The likelihood of a genetic exchange from other organisms is described in paragraph 1.1.5 of the Environmental Risk Assessment. The overall conclusion of the calculated risk of such an event was extremely low.

(d) likely consequences of gene transfer:

The likelihood of a gene transfer is described in paragraph 1.1.5 of the Environmental Risk Assessment. The overall conclusion of the calculated risk of such an event was extremely low.

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.):

The behaviour and characteristics of the GMO was compared with the wild type FHV in survivability studies on a non-porous surface, in kitty litter and in water. Studies are described in paragraph 3.4 of the Environmental Risk Assessment. The overall conclusion was that the behaviour of the GMO was not changed compared with the wild type strain.

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

… Methods for tracing: culture on suitable cell lines and PCR.

Monitoring: The trial will be conducted according to GCP guidelines. Clinical examination of the recipient animal will be performed.

2. Methods for monitoring ecosystem effects
No specific monitoring of ecosystems will be monitored.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Transfer of the donated genetic material from the GMO to other organisms can be detected by PCR.

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

5. Duration of the monitoring
Cats will be vaccinated twice with a 3-4 week interval. After the second vaccination cats will be observed for another two weeks. All cats will not be vaccinated at one day but in a timescale of 1-4 months. So the total duration of the monitoring is approximately 5,5 months. Every single cat will be observed for 6 weeks, from the first day at vaccination till 2 weeks after the second vaccination.

6. Frequency of the monitoring
Cats will be monitored by a veterinarian three times (first and second vaccination and two weeks after second vaccination) and on a daily basis by the owner.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
No specific requirements are taken to disinfect the site of introduction after the trial. After each vaccination of a cat the surroundings of the place where the cat will be vaccinated, will be disinfected according standard practice …

2. Post-release treatment of the GMOs
The GMO's will no longer persist in the cat and the environment.

3. (a) Type and amount of waste generated
Empty vials, applicator. One vial will contain one dose of vaccine. The number of vials and applicators therefore depends on the number of cats that will be vaccinated.

3. (b) Treatment of waste
The empty vials and applicator will be collected at each study site by the Sponsor and will be destroyed internally at the Sponsors R&D site according the internal procedures for recombinant products

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

If there is an indication that there is an unexpected spread, oropharyngeal swabs from vaccinated cats can be taken to check whether these cats still shed the vaccine strain. It these swabs are positive it will be recommended to keep these cats indoor for 3 weeks. Thereafter new swabs will be taken to determine if the vaccine strains are still shedding.

2. Methods for removal of the GMO(s) of the areas potentially affected
The vaccine strains survive for approximately three weeks in a cat and onkly for a very short period (hours) in the environment. Removal of the areas potentially affected by the GMO can be preferably be done by preventing the spreading from cat to cat by keeping vaccinated cats indoor.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
The vaccine strains will not survive in the environment and can only infect cats. After an infection the vaccine strain will disappear within approximately 3 weeks in the infected cat.

4. Plans for protecting human health and the environment in the event of an undesirable effect
Despite the negligible risk related to the use of Pfizer FIV an emergency plan is established. In case of accidental injection to humans we recommend to seek medical advice immediately and show the package insert or the label to the physician. In the case of accidental breaking of a vial the contaminated surface should be disinfected with 10% chlorine bleach.

In case of an unexpected event, 3 operating phases are implemented:

-Alert phase
Any observation which cannot be related to the normal post vaccinal adverse reactions (and transient lethargy) must be reported to the investigator veterinary surgeon and to the monitor of the trial.
The concerned animal will be kept indoors by its owner.

-Investigation phase
Appropriate samples are collected and sent to the laboratory for virus isolation and identification.
Treatment of the animal is immediately prescribed by the veterinary surgeon.

-Action phase
The diagnosis is known before the end of the trial and the event is not related to the vaccine: The investigator starts treating the concerned animal

The diagnosis is known before the end of the trial and the event is related to the vaccine: The recruitment of cats for the trial is stopped. Owners of cats which have already been vaccinated with Pfizer FIV vaccine are asked to keep their cats indoors for a 1 month follow up.
The cause of the event is not known before the end of the trial.
If the cause of the unexpected event is not established at the end of the trial, an adverse reaction related to the vaccine cannot be eliminated. The follow up of all the animals included in the trial will be extended for 1 month after the trial.