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	**the Netherlands**
(b)	Notification number	**B/NL/10/002**
(c)	Date of acknowledgement of notification	**04/02/2010**
(d)	Title of the project	**Vaccination with naked DNA, encoding the xenogenetic human tyrosinase for the induction of specific defence against melanoma cells in dog**
(e)	Proposed period of release	**From 01/01/2011 until 31/12/2015**

2. Notifier

Name of institution or company:

Utrecht University,
Faculty of Veterinary Medicine
Dep. Clinical Sciences of Companion Animals
Yalelaan 108
3584 CM Utrecht,
PO Box 80.154
3508 TD Utrecht
The Netherlands

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class

The medicinal product is not a GMO. The investigational gene therapy medicinal product is a plasmid, pING/Tyrosinase. It is a closed circular DNA molecule, without the properties of a living organism or virus.

(b) Identity of the GMO (genus and species)
Not relevant. The plasmid DNA does not have a taxonomic classification

(c) Genetic stability – according to Annex IIIa, II, A(10)
Annex IIIa, II, A(10): Verification of the genetic stability of the organism and factors affecting it. **Available data indicates the prokaryotic and eukaryotic sequence elements of the pING/Tyrosinase are genetically stable under the controlled conditions used, and not become unstable via integration of plasmid sequences.**

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	(**X**)	No	(.)

If yes:
- Member State of notification **USA**
- Notification number B/../../… **unknown**

The production of the plasmid is performed by Merial Inc., USA, and is not part of this notification. During production the plasmid-batch will be subject to several general accepted quality tests to guarantee identity, purity and quality of the product. The product is guaranteed free of bacterial contamination.

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

The plasmid pING/Tyrosnase is a double strand, covalently closed circular DNA molecule which has no properties of a living organism or virus and cannot be considered as a GMO. After vaccination, the dog carries a number of genetic modified cells. These cells cannot survive the environment outside the dog.
In the plasmid pING/Tyrosinase is an immediate early promoter from the HCMV virus present. The HCMV virus is a herpes virus and infection with these viruses occurs mainly via oral and sexual routes, by which principally epithelia cells will be infected.

The host range for HCMV is restricted to humans. An infection in dogs with HCMV has never described.
There is no reason to assume that transmission of the plasmid DNA in the reproductive path will occur, because of the way of administration, the plasmid will be transient occur in muscle cells and possible in some skin cells. Plasmid DNA that enters the blood will be decomposed by nucleases.
Theoretically an interaction of the plasmid with a virus or viral sequence in the cell is possible. The chance is however negligible small.
Also the chance that there will be an uptake of the plasmid by bacteria, whereby bacteria become GMO and facilitate replication of the plasmid will be negligible small.

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 (.)
bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …

other, specify …

pING/Tyrosinase is commercially available pDNA from Merial Inc., USA. This plasmid is specially designed for treating melanoma tumours in dogs and used over 9 years in the USA. This artificial bacterial plasmid does not exist in the natural environment and does not have a taxonomic classification.

2. Name

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

3. Geographical distribution of the organism

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

pING/Tyrosinase is not an organism but a plasmid vector. The pING/Tyrosinase is used in the USA as a medicinal treatment in dogs.

(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 (.)

Not applicable. The plasmid is used in animal health care in the USA

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (.)
Not applicable. The plasmid is used in animal health care in the USA

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (.)
Not applicable. The plasmid is used in animal health care in the USA

4. Natural habitat of the organism

(a) If the organism is a micro organism

water (.)
soil, free-living (.)
soil in association with plant-root systems (.)
in association with plant leaf/stem systems (.)
other, specify …
pING/Tyrosinase is not an organism but a plasmid vector. The plasmid is not known to exist in the natural environment

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

5. (a) Detection techniques
Polymerase Chain Reaction (PCR) assays.

(b) Identification techniques
Plasmid identity has to be confirmed via enzymatic restriction mapping and agar gel electrophoresis. PCR assays.

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
…

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

pING/Tyrosinase is not an organism but a plasmid vector. The plasmid is not known to exist in the natural environment, so generation time in natural ecosystems is not known.

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

pING/Tyrosinase will not undergo episomal replications in mammalian cells.

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

(d) Factors affecting reproduction:
The pING/Tyrosinase is capable of reproduction (replication) in bacteria. Because the plasmid has a ColE1 ori for replication, but only bacteria of the family *Enterobacteriaceae* are capable to facilitate replication of plasmid DNA. These bacteria are not common on the skin and in muscles of canine where the plasmid will be injected. In the unlikely case the plasmid will be in contact with bacteria from the intestine the uptake of the plasmid in the absence of kanamycin or neomycin will have no advantage for growth and will lead to loss of the plasmid.
Incorporation of plasmid by bacteria is an inefficient process and only a small part of bacteria species are competent under specific conditions.

9. Survivability

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

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (fungi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	…

(x)
pING/Tyrosinase is not known to form any of the structures listed above. Instances of inadvertent environmental release would generally be expected to lead to rapid degradation of the plasmid due to the abundance of deoxyribonucleases in the natural environment

(b) relevant factors affecting survivability:

Because the plasmid has a ColE1 ori for replication, only bacteria of the family *Enterobacteriaceae* are capable to facilitate replication of plasmid DNA. The change that the plasmid will be taken up by bacteria and will be persistent is negligible small.

10. (a) Ways of dissemination

Theoretically an interaction of the plasmid with a virus or viral sequence in canine is possible. The chance is however negligible small.
Also the chance that there will be an uptake of the plasmid by bacteria, whereby bacteria become GMO and facilitate replication of the plasmid will be negligible small.
Finally is the change that vertical transmission will occur is negligible small.

(b) Factors affecting dissemination

An immediate early promoter from the HCMV virus is present in the plasmid pING/Tyrosinase. The HCMV virus is a herpes virus and infection with these viruses occurs mainly by oral and sexual routes, by which principally epithelia cells will be infected. The host range for HCMV is restricted to humans. An infection in canine with HCMV has never described.
HCMV has no tropism for keratinocytes and therefore will not be present on the skin where the plasmid will be intramuscular administered by a Bio injector.

Because the plasmid has a ColE1 ori for replication, only bacteria of the family *Enterobacteriaceae* are capable to facilitate replication of plasmid DNA. These bacteria are not common on canine skin or in muscle cells.
The plasmid will be intramuscular administered by a Bio injector. (This is a needle free system, whereby fluid under CO2-pressure will be administered into the muscle) The change that injected plasmid will be transported to the germinal cell is negligible.

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)

None

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

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

The pING/Tyrosinase is a commercially available plasmid in the USA. It has an immediate early promoter sequence from HCMV, CMV exon 1 and 2, CMV intron A, a kanamycin resistance cassette, a bovine growth hormone (BGH) Transcriptional Terminator, a pUC origin of replication site for the propagation of the plasmid in E.coli and a human Tyrosinase gene. Human tyrosinase will be expressed. Tyrosinase catalyses the conversion from tyrosine to skin pigment melanin. By immunisation of xenogenetic (human) tyrosinase an immune response against endogenous (canine) tyrosinase is induced. Recognition by the immune system of malign melanoma cells results in removal of the tumour.

3. (a) Has a vector been used in the process of modification?

Yes (.) No (**X**)

If no, go straight to question 5.

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

Yes (.) 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	(.)
cosmid	(.)
transposable element	(.)
other, specify …	

(b) Identity of the vector

…

(c) Host range of the vector

…

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

Yes (.) No (.)

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted
…

(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 …

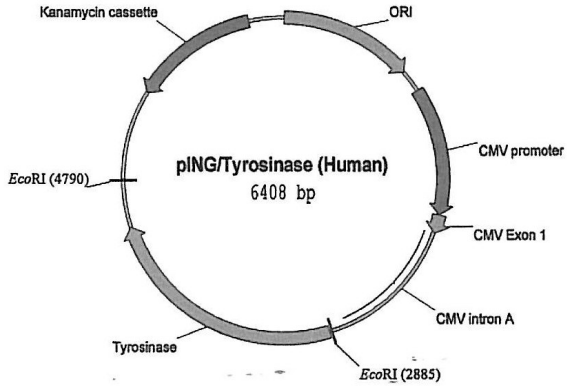
5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

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

pING/Tyrosinase was constructed using standard recombination DNA cloning methods.

6. Composition of the insert

(a) Composition of the insert



In the multiple cloning site has been cloned the sequence for human tyrosinase.

(b) Source of each constituent part of the insert

Tyrosinase is human tyrosinase

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

The plasmid expresses the human Tyrosinase protein. By this immunisation with xenogenetic tyrosinase (human), an immune response against endogenous canine tyrosinase will be generated. Melanocytes and malign melanoma cells express tyrosinase. The immune system will recognize due to the autoimmune response the endogenous tyrosinase in the malign melanoma and eliminate the tumour cells.

(e) Location of the insert in the host organism

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

(f) Does the insert contain parts whose product or functions 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		(.)	
DNA virus		(.)	
bacterium		(.)	
fungus		(.)	
animal			
-	mammals	**(X)**	**in vitro gene (DNA) of human tyrosinase**
-	insect	(.)	
-	fish	(.)	
-	other animal	(.)	
	(specify phylum, class)	…	
other, specify		…	

2. Complete name

(i)	order and/or higher taxon (for animals)	**Animalia**
(j)	**phylum**	**Chordata**
(k)	**class**	**Mammalia**
(l)	**order**	**Primates**
(ii)	**family ~~name for plants~~**	**Hominidae**
(iii)	**genus**	**Homo**
(iv)	**species**	**H. sapiens**
(v)	subspecies	…
(vi)	strain	…
(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 (.) No **(X)** Not known (.)

If yes, specify the following:

(a) to which of the following organisms:

humans	(.)
animals	(.)
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 (.) Not known **(X)**

Specify …

pING is not a GMO. Survival of pING pDNA in the natural environment has not been studied. Based on data and considerations under B(8) and B(9) above, the potential for survival in the natural environment as a consequence of the intended use is considered negligible.

(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 …

pING is not a GMO. Because pING has a ColE1 ori of DNA replication from bacterial origin (E.coli), only bacteria of the familiy *Enterobacteriaceae* are capable to facilitate the replication of the plasmid DNA.

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

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

Specify …

pING is not a GMO. Dissemination of pING in the natural environment has not been studied. Based on data and considerations provided under B(8) and B(9) above, the dissemination of this plasmid in the natural environment as a consequence of the intended use is considered negligible.

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

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

Specify …

pING is not a GMO and not pathogen in dogs.

2. Genetic stability of the genetically modified organism

Not applicable. pING is not a GMO

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
Polymerase Chain reaction (PCR) assays.

(b) Techniques used to identify the GMO
Polymerase Chain reaction (PCR) assays.

F. Information relating to the release

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

The purpose of the release is a clinical trial in dogs with metastasized skin melanoma, which are finished with traditional treatments.

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 …

Not applicable. There is not natural habitat or ecosystem for the pING plasmid.

3. Information concerning the release and the surrounding area

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

Utrecht University,
Faculty of Veterinary Medicine
Dep. Clinical Sciences of Companion Animals,
Yalelaan 108,
3584 CM Utrecht.

(b) Size of the site (m^2): … m^2
(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

Not relevant, see below.

(e) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not relevant, see below.

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

Sections (b)-(d) are not considered relevant. Environmental release of pING is not intended beyond the treatment of trial subjects.
Subjects will be administered the product by the needle free system of Bio injection into muscular tissue of paws. Treatment will be given at the Utrecht University, Veterinary Medicine facilities of department of Clinical Sciences of Companion Animals, on an outpatient basis.
Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs) cannot be specified under these circumstances as the movement of treated subjects will not be restricted. Flora and fauna including crops, livestock and migratory species which may potentially interact with pING pDNA under these circumstances include bacteria present on or in canine subjects as detailed under B(8)(d) above.

pING is not a GMO.

4. Method and amount of release

(a) Quantities of GMOs to be released:

Patients (canine) will receive multiple injections of the plasmid (not a GMO) with a needle free system (Biojector 2000). Vaccination will be done at a fortnight interval, with a maximum of 4 injections. After that, depending on the progress of the tumour in the dog, booster injections with 6 months intervals can be given, with a maximum of 4 boosters.
The dose of the injection will be always 500 µg of plasmid DNA.

(b) Duration of the operation:

The duration of this clinical trial will not exceed 5 years.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
Safety containment measures for handling of the pING plasmid will follow institutional guidelines.
Import and quality controls of the vaccine will be performed under responsibility of the Pharmacy of the faculty of Veterinary Medicine.
Patients (canine) will be isolated from other animals during treatment. This facility meets the safety containment measures for animals in combination with GMO. This exceeds the safety level, because pING is not a GMO.
Owners of the patient will not be present during treatment.

5. Short description of average environmental conditions (weather, temperature, etc.)

The plasmid will be administered at the Utrecht University, faculty of Veterinary Medicine in an enclosed facility at ambient temperature.

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.

Since the launch of the melanoma vaccine (pING/Tyrosinase) in the US, approximately 6000 dog patients have been treated without reported recombination events. Also with similar vaccines licensed by Merial Inc, USA, no recombination or environmental impact have been reported.
These vaccines are not GMO's.

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)	…
(ii)	family name for plants	…
(iii)	genus	…
(iv)	species	…
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

Human:

Kingdom:	**Animalia**
Phylum:	**Chordata**
Class:	**Mammalia**
Order:	**Primates**
Family:	**Hominidae**
Genus:	**Homo**
species:	**H. sapiens**

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

The pDNA will be injected into the muscles of the dog. Muscles cells are the major cell type to be transfected. Based on pre-clinical data, gene expression by transfected cells will be transient and most likely short (several days). Despite the short duration of gene expression, preclinical and clinical data have revealed that this pDNA vaccination strategy is potent and fast in the induction of immune responses against endogenous canine tyrosinase.
In any case that pING can reach the blood; it will be destroyed by deoxynucleases.

3. Any other potentially significant interactions with other organisms in the environment

Other potentially significant interactions with other organisms in the environment are not expected for the reasons given under B(8) and B(10) above.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

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

Give details

See B(8) and B(10) above.

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

The pING pDNA (not a GMO) could potentially disseminate into soil and waste water trough accidents in transport or improper handling and disposal at the clinical site. The possibility of dissemination and establishment in soil or (waste)water ecosystems (e.g. uptake and replication in permissive bacteria) is considered remote based on the reasons given under B(8) and B(10).

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

(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	…

No significant harm to non-target organism is anticipated.

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
The likelihood of genetic exchange between pING and bacteria present on or in canine subjects is considered remote; as stated under B(8).

(b) from other organisms to the GMO:
Not relevant, because plasmid is not a GMO.

(g) likely consequences of gene transfer:
Because the plasmid has a ColE1 ori for replication, only bacteria of the family *Enterobacteriaceae* are capable to facilitate replication of plasmid DNA. The change that the plasmid will be taken up by bacteria and will be persistent is negligible small. See also B(8), B(9), B(10).

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.):

No studies of pING have been performed in simulated natural environments.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

No significant interactions with biogeochemical processes are anticipated.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
   The plasmid DNA (pING) will be monitored by sampling blood from the patient (canine) to detect plasmid DNA in the patient. Considering the abundance presence of deoxynucleases in the blood, the expectation to find pING is very low.

2. Methods for monitoring ecosystem effects
   …
3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
   …
4. Size of the monitoring area (m^2)
   … m^2
5. Duration of the monitoring
   …
6. Frequency of the monitoring

   Methods for monitoring include PCR assays as have been developed for testing the quality of the plasmid. Except for monitoring in patient's blood and based on the clinical safety experience no monitoring in the ecosystems is planned.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

After vaccination the site, the direct environment of the dog (patient) and on the dog will be checked on plasmid fluid present.
The room will be cleaned according to the standard procedure with cleansing and disinfection agents.

2. Post-release treatment of the GMOs

A post-injection clinical evaluation of the trail subjects (vital signs and symptom-directed exam) will be performed after each administration. The subject may leave the clinic once the investigator deems the subject to be clinically stable.
The plasmid DNA (pING) will be monitored in the animal by sampling blood from the patient (dogs) to detect plasmid DNA. Standard PCR assay on human tyrosinase will be used.
Disposal of used materials (e.g. gloves, vials, tissues, and etcetera) that may potentially contain residual amounts of vaccination product will be performed in accordance with legal and institutional procedures of the Utrecht University.

3. (a) Type and amount of waste generated

The type of waste will be used syringes (without a needle!), vials, gloves and tissues or other disposables that may potentially contain residual amounts of the medicinal product. The waste generated on a daily basis will not exceed 0.5 mg pDNA.

3. (b) Treatment of waste

Treatment of medical and GMO waste will be performed in accordance to legal and institutional procedures of the Utrecht University.

J. Information on emergency response plans

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

   The treatment of the canine patients will be performed in a DM-II facility room for handling animals in combination with GMO's. This room has a faeces and urine collector to prevent uncontrolled spread into the environment.
   Instructions on spill response procedures will be provided to the clinical site. Site personnel responsible for spill cleanup will be instructed to wear proper personal protective equipment (protective gloves, clothes).

2. Methods for removal of the GMO(s) of the areas potentially affected

   Spilled liquids will be absorbed with common absorbent materials and placed in appropriate waste containers.

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

   Disposal or sanitation of plants, animals (other than the patients), soils, etc. exposed to the product is not mandated due to the low risk of resultant harm.

4. Plans for protecting human health and the environment in the event of an undesirable effect

   Because the medicinal product does not appear to pose significant risk to human health or the environment, specific plans for protection have not been prepared.