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/16/013
(c)	Date of acknowledgement of notification	13/02/2017
(d)	Title of the project	

Clinical evaluation of a live attenuated Respiratory Syncytial Virus (RSV) vaccine based on a genetically modified RSV virus lacking the coding sequence for the attachment protein G

(e)	Proposed period of release	*From 16/04/2018 until 31/12/2030*

2. Notifier

Name of institution or company: *Centre for Human Drug Research*
Zernikedreef 8
2333 CL Leiden
The Netherlands

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 *Paramyxoviridae, class V (negative sense single-stranded RNA virus)*

(b) Identity of the GMO (genus and species)
Pneumovirus, Respiratory Syncytial virus A

(c) Genetic stability – according to Annex IIIa, II, A(10)
The active ingredient in the RSVΔG and G-RSVΔG candidate vaccines is a live GMO RSV. The genetic modification of this GMO consists of a gene deletion and is therefore a stable mutation. The deletion of the G gene attenuates the virus. In the absence of the attachment protein G the infectivity and thereby replication of RSV is strongly reduced. It is highly unlikely that RSVΔG will regain the genetic material encoding the G protein through recombination, since this is very rare for negative sense single stranded RNA viruses. If recombination should occur, the progeny would be intermediate attenuated or similar to the wildtype RSV that is ubiquitously present in the environment.

Genetic stability of additional minor modifications during production was confirmed by sequencing the genome of the pre-seed (original GMO virus),the working seed lot and a vaccine batch. Genetic stability of RSVΔG after administration will be studied by deep-sequencing during the clinical trial if RSVΔG can be recovered from clinical specimens.

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.

Humans are the only natural host and the only natural reservoir of RSV. It is highly unlikely that other organisms will be affected by RSVΔG. Survival of RSVΔG and natural RSV in the environment is short-lived.

The genetic modification of RSVΔG consists of a gene deletion. RSVΔG does not contain inserted genes; therefore gene transfer of inserted genes is not possible. RSV itself is not invasive and not persistent. Because the genetic material for the attachment protein G in the RSVΔG virus is deleted, infectivity and replication of the virus in the host is impaired. Concept vaccine G- RSVΔG consists of RSVΔG virus that is complemented with G protein via culture on cells expressing the membrane-bound form of the G protein. G-RSVΔG expresses G proteins on its surface while the viral genome does not contain the gene for the G protein. G-proteins are only present for the first round of infection. Newly formed virus particles (progeny) will be RSVΔG lacking the G protein and these particles are therefore less efficient in infecting host cells and are less likely to spread within and between hosts.

The GMO is intended for immunization of humans against natural occurring RSV. Immunization with the GMO is expected to be without harmful effects. Due to the attenuation, and because most humans have pre-existing immunity against RSV, it is unlikely that (G-)RSVΔG will cause a measurable infection in adults. Exposure of healthy adults to wild-type-RSV may cause a mild infection of the upper respiratory tract with symptoms of RSV-infection, such as rhinorrhea, pharyngitis, sneezing, and cough. Pathogenicity of the GMO is expected to be less than that of wild-type RSV. If infection with the GMO occurs, it is most likely to be asymptomatic. If infection becomes symptomatic, symptoms are expected to be mild. In addition, the GMO will induce immunity against natural occurring RSV.

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	(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	*Orthopneumovirus*
(iii)	species	*Human Respiratory Syncytial virus*
(iv)	subspecies	*Subtype A*
(v)	strain	*Clinical isolate RSV 98-25147-X (Netherlands 1998)*
(vi)	pathovar (biotype, ecotype, race, etc.)	*genotype GA2*
(vii)	common name	*Human Respiratory Syncytial 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 (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	humans (respiratory tract)

Humans are the natural hosts

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

Not applicable

5. (a) Detection techniques

Q-PCR and quantitative culture

(b) Identification techniques

Q-PCR, sequencing

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

*Paramyxoviridae viruses and human RSV are categorized as class 2 pathogen according to the Dutch regulations "*the Regeling GGO 2013 Appendix 4.1*"*

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	(X)
animals	(.)
plants	(.)
other	(.)

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

Respiratory syncytial virus (RSV) is one of the most common lower respiratory diseases in infants and young children worldwide. The majority of children and adults display a mild illness of the upper airways; however, 2–5% of infected infants and young children will develop a severe bronchiolitis, which requires hospitalization. These patients have increased risk to suffer recurrent wheeze and asthma. RSV can also cause severe lower respiratory tract infections in elderly especially in the presence of comorbid conditions such as chronic obstructive pulmonary disease and congestive heart failure.

Humans are the only natural reservoir of RSV. RSV primarily infects human epithelial cells within the nasopharynx. The infectious dose for RSV is > 160 - 640 CCID50, administered through intranasal spray.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Viral load of natural occurring RSV in healthy human adults peaks at 5-6 days post infection (DeVincenzi, Am J Respir Crit Care Med. 2010 Nov 15; 182(10): 1305–1314). Incubation period is 2-8 days. Generation time of RSVΔG and G-RSVΔG remains to be determined.

(b) Generation time in the ecosystem where the release will take place:
Same as in (a).

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

(d) Factors affecting reproduction:

Infectivity, dissemination and host immune response affect the reproduction of the virus. RSVΔG and G-RSVΔG lack the genetic material for the G attachment protein and is therefore strongly impaired in its infectivity and replication.

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:

RSV is vulnerable to environmental changes, such as high and low temperature, pH and low humidity levels.

10. (a) Ways of dissemination

Humans are the only source of infection. The virus is believed to spread primarily via droplets (via sneezing) at short distances (typically <1-2 meter) or fomites. Direct contamination has a higher impact than airborne transmission. The eyes and nose are the most sensitive inoculation areas

(b) Factors affecting dissemination

The virus can survive on non-porous surfaces for many hours and for 30 minutes or more on body parts. Close person-to-person contact or contact with contaminated environmental surfaces and autoinoculation are required for transmission. Proper handwashing techniques and keeping the hands away from the eye and nose areas can assist in decreasing transmission. The period of viral shedding of wild-type RSV usually is 3 to 8 days but may

last longer, especially in young infants and in immunosuppressed people, in whom shedding may continue for as long as 3 to 4 weeks.

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

In summary, the original and clinical vector deviate from the original virus in the following ways:

- *RSVΔG contains three restriction sites in the non-coding regions*
- *The G protein coding sequence including gene start and gene end is deleted. At the excision site the restriction sites BsiW I and BssH II re-ligated.*
- *Two point mutations due to primer design mismatch*
- *Seven spontaneous point mutations have occurred: four outside open reading frames (ORF), one silent point mutation in the ORF of nonstructural protein 1 (NS1) and two point mutations in the ORF of F-protein leading to amino acid changes.*

2. Intended outcome of the genetic modification

RSVΔG is a genetically modified RSV virus designed for use as a prophylactic life-attenuated viral vaccine against natural RSV. The genetic modification involves deletion of the gene for the G attachment protein. As a result of the deletion of the genetic material for the G attachment protein, RSVΔG particles lack the G protein. G-RSVΔG vaccine is based on the same virus (RSVΔG) but is complemented with the G protein by culturing on cells expressing the membrane-bound form of G. The resulting G-RSVΔG expresses G proteins on its surface while the viral genome does not contain the gene for the G protein. This only accounts for the first round of infection. Newly formed RSV particles (progeny) will be RSVΔG lacking the G protein.

The G protein of RSV is associated with attachment to the cells and replication of RSV. In absence of G protein, attachment of RSV to the cells of the nasopharynx is impaired and virus replication is reduced. In addition, the G protein of RSV is associated with immune evasion, which is one of the causes of its pathogenicity. Thus as a result of the genetic modification RSVΔG and G-RSVΔG have an attenuated phenotype.

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 (.) No (X)

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 C.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

NA…

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

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

(a) Location of the insert in the host organism

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

(b) Does the insert contain parts whose product or function are not known?
Yes (.) No (.)
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 (.)
- 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 …

(iv) species …
(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 (.) 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 (.) 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 (.)
If yes, specify …

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) No (.) 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 (X) No (.) Unknown (.)
Specify

RSVΔG and G-RSVΔG are attenuated by deletion of the gene for the G attachment protein. In the absence of the G attachment protein on the vaccine (RSVΔG) and/or its progeny (RSVΔG and G-RSVΔG) infectivity and replication of the virus in vivo is strongly diminished.

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

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

Specify

RSVΔG and G-RSVΔG are attenuated by deletion of the gene for the G attachment protein and are not able to produce virus containing G protein . In the absence of the G attachment protein, infectivity of the virus is strongly diminished, limiting dissemination to other cells and hosts.

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

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

Specify

RSVΔG and G-RSVΔG are less pathogenic due to its reduced infectivity.

2. Genetic stability of the genetically modified organism

The genetic modification consists of a gene deletion and is therefore a stable mutation. Genetic stability of additional minor modifications during production was confirmed by sequencing the genome of the pre-seed (original vector), the working seed lot (used as starting material in the production of both RSVΔG and G-RSVΔG vaccine) and a batch of G-RSVΔG vaccine).

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	(X)
animals	(.)
plants	(.)
other	…

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

The GMO was non-pathogenic in cotton rats and Wistar rats. Replication and shedding of the GMO in the cotton rat model was below the detection limit of the CCID50 assay (2 log10/ml). No toxicity or allergenic effects of the GMO were found in a single and repeated dose toxicity and local tolerance test in Wistar rats.

Information on pathogenicity of RSVΔG and G-RSVΔG vaccine in humans is not available and is the objective of the proposed study. The RSVΔG and G-RSVΔG

vaccine are intended for immunization of humans against natural occurring RSV. Immunization with RSVΔG or G-RSVΔG is expected to be without harmful effects. Due to the attenuation, and because most humans have pre-existing immunity against RSV, it is unlikely that RSVΔG or G-RSVΔG vaccine will cause a measurable infection in adults. If infection with RSVΔG or G-RSVΔG occurs, it is most likely to be asymptomatic, but symptoms of an upper respiratory tract infection may occur, such as rhinorrhea, pharyngitis, sneezing, and cough. Because of the attenuated phenotype of RSVΔG and G-RSVΔG, symptoms caused by infection with either RSVΔG or G-RSVΔG, if any, are expected to be mild. In addition, the GMO will induce immunity against natural occurring RSV.

RSV is not a persistent or invasive virus in itself, and it is therefore highly unlikely that the attenuated virus (RSVΔG) becomes persistent and invasive. The infective dose of the RSVΔG and G-RSVΔG vaccine remains to be determined but is expected to be at least a factor 100 higher than the parental virus. The host range has not changed.

Recombination of the RSVΔG virus with wildtype RSV is highly unlikely. The probability of RSVΔG recombining with other virus strains is unlikely. RSV is a negative stranded non-segmented RNA virus for which recombination seems to be generally rare or even absent. Being a non-segmented virus, it cannot recombine through re-assortment like influenza viruses. In this light, it is highly unlikely that RSVΔG will regain the complete genetic material for the G protein through recombination.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Quantitative PCR and Quantitative culture

(b) Techniques used to identify the GMO
Quantative PCR, sequencing when possible

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 human clinical trial to investigate safety and preliminary immunogenicity in human subjects of RSVΔG candidate vaccine or RSVΔG complemented with G protein via culture on cell substrate expressing the membrane form of G protein (G-RSVΔG) candidate vaccine.

RSVΔG and G-RSVΔG vaccine are intended to be used as a prophylactic vaccine against lower respiratory tract infections (pneumonia, bronchitis, etc) caused by RSV. Clinical trials in adults are needed to support further clinical development towards a prophylactic vaccine to protect infants and young children and possibly elderly, against lower respiratory tract infection caused by RSV. Vaccination against

RSV will reduce the number of acute respiratory tract infections, RSV related hospitalizations and deaths.

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):

Leiden, the Netherlands

(b) *Not applicable*

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

The vaccine will be administered by intranasal spray in a dedicated patient room at CHDR. This room has nothing but smooth surfaces (no carpeting, wallpaper and curtains), which are easy to clean / disinfect. The room holds only a bed, a table and a chest of drawers with necessary materials.

(a) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:

Not applicable

(b) 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:

Humans will receive a single dose of RSVΔG and G-RSVΔG containing 4-11 log_{10} $CCID_{50}$. In the project up to 600 subjects will participate in clinical trials with RSVΔG and/or G-RSVΔG vaccine. Subjects will receive either a placebo or a preparation of either RSVΔG or G-RSVΔG vaccine. The quantities that will be released in the environment by the subjects will be very small because of the reduced infectivity of the attenuated virus.

(b) Duration of the operation:

Each subjects will receive a single dose of the vaccine by nasal spray. Shedding of the RSVΔG virus will be monitored 2-4 weeks or until no longer detectable in nasal samples.

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

- *The attenuated phenotypes of RSVΔG and G-RSVΔG vaccines will minimize the spread of RSVΔG virus. In addition the following methods and procedures are in place:*
- *Only healthy adults are included in the study, with no known or suspected immune deficiency or disease that may influence the immune system, no use of medication that may influence the immune system*
- *in the first in human trials study subjects should not have close contact with children younger than 2 years or immune-compromised individuals during two weeks after administration of the vaccine.*
- *Reconstitution and/or dose preparations (if applicable) are performed in a biological Class II safety cabinet in a cleanroom according to MLII requirements (Regeling GGO 2013, Appendix 9.1.1.3.2) of the IGFL facility of the LUMC.*
- *Personnel involved in the clinical procedures will wear protective clothing and gloves*
- *All disposables that may have been in contact with the GMO will be discarded as clinical waste in UN3291 containers.*
- *Biological samples will be transported according to Regeling GGO 2013, appendix 1, paragraph 1.1 (internal transport) or will be in accordance with the European Agreement concerning the international carriage of Dangerous goods by Road UN3373 (ADR classification UN3373 and shipping instructions) or the ICAO Technical Instructions when air transported is involved (external transport).*
- *In case of spills of vaccine or biological samples the spill will be absorbed and treated with 1000 ppm chlorine solution and the contaminated area will be disinfected with 1000 ppm chlorine solution.*
- *Preparation and administration of the vaccine will be performed in the same room at CHDR. This room has nothing but smooth surfaces (no carpeting, wallpaper and curtains), which are easy to clean / disinfect. The room holds only a bed, a table and a chest of drawers with necessary materials*
- *To eliminate air and to ensure desired volume is contained within the nozzle and the syringe the plunger is depressed until the prongs of the dose divider clip will impact the finger-flange of the syringe. During this procedure, the nozzle of the device will be held on a gauze in a tube to avoid the release of aerosols in the room.*
- *After administration, all components will be discarded as clinical waste into the appropriate UN3291 container. Fluid that runs out of the nose directly after administration will be removed with facial tissues that will be discarded as clinical waste into the appropriate UN3291 container.*

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

The studies will be performed at in the Netherlands which has a temperate maritime climate with cool summers and moderate winters. The preparation and administration of the vaccine will take place in environmentally controlled hospital rooms (ambient indoor conditions for administration).

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 available. No clinical trials have been performed with RSVΔG and G-RSVΔG vaccines yet.

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

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

Activation of immune responses in vaccinated subjects resulting in protection against severe lower respiratory tract infection by RSV.

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

None expected

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

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

Give details

RSVΔG and G-RSVΔG are attenuated by the deletion of the gene for the G attachment protein. In the absence of the G attachment protein infectivity and replication of the virus is strongly diminished.

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

Humans. Humans are the only natural reservoir for RSV and the genetic modifications in RSVΔG or G-RSVΔG do not affect the host range.

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, the genetic modification consist of a gene deletion.

(b) from other organisms to the GMO:

Negligible. RSV is a negative stranded non-segmented RNA virus for which recombination seems to be generally rare or even absent.

(c) likely consequences of gene transfer:

Genetic exchange between natural RSV and the GMO is highly unlikely, but in the case this occurs, the result will be a less attenuated GMO that resembles more the natural RSV that has co-infected the host. No consequences for the organism and the environment are expected.

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 RSVΔG is studied in cotton rats (Widjojoatmodjo, Boes et al. 2010). G-RSVΔG are *is also tested in cotton rats (unpublished data) with comparable results.*

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

No

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Quantitative culture and quantitative PCR on nasal washes or swabs from clinical trial subjects.

2. Methods for monitoring ecosystem effects

Not required.

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

Not applicable. The GMO does not contain inserted genes.

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

Not applicable

5. Duration of the monitoring

Shedding of RSVΔG in biological samples will be monitored at selected time points until 2-4 weeks after administration. If shedding of RSVΔG is detected at any time point, the individual will be monitored until shedding is absent.

6. Frequency of the monitoring

Shedding of RSVΔG will be monitored in nasal wash or swabs at 2-5 time points after administration of the vaccine or until shedding is no longer detected.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

All materials, equipment and table tops that have been in possible contact with the biological samples will be disinfected with 1000 ppm chlorine solution (cleaning materials such as paper towels will be discarded as clinical waste in UN3291 containers). Spills will be disinfected using 1000 ppm chlorine solution. Used material will be discarded as clinical waste, UN3291. When the subject is discharged, the possible contaminated surfaces will be cleaned hygienically.

2. Post-release treatment of the GMOs

All unused and used material containing the GMO present at the site will be discarded as clinical waste (UN3291).

3. (a) Type and amount of waste generated

The following materials may be used during the study that may be in contact with the GMO:
- *Vials, syringe, needle and nasal administration device*
- *Gauzes or tissues*
- *Gloves*

- *Disposable lab coats, if used*
- *Collection tubes and containers for biological samples and other disposables used for collection of biological samples*
- *Disposable pipets*
- *Petri dishes*

3. (b) Treatment of waste

Used waste that has potentially been exposed to the GMO during all procedures will be collected as clinical waste and will be discarded in a, UN3291 certified container.

J. Information on emergency response plans

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

In case of accidental spillage of the vaccine or biological samples possibly containing the vaccine (e.g. on the workbench or on the floor), local procedures will be followed to contain and immediately disinfect the spill to prevent further spread. In brief:

1. Put on appropriate protective clothing and equipment
2. Lay absorbing material around the spill to prevent further spreading
3. Soak absorbent material with 1000 ppm chlorine solution and leave for at least 5 min
4. Wipe up the spill with absorbent material, starting from the outside and moving inwards
5. Remove the absorbing material
6. Soak absorbent material with 1000 ppm chlorine solution
7. Disinfect the contaminated area with the absorbent material soaked in 1000 ppm chlorine solution
8. Stainless steel surfaces will be subsequently rinsed with water to prevent corrosion of the surface.
9. Discard all materials used in the spill clean-up as clinical waste, UN3291

Accidental exposure of health care professionals to the vaccine or biological samples possibly containing the vaccine should be treated according to the following measures:

- *Needle-stick injury: Encourage bleeding of the wound. Wash injection area well with water and physiological salt solution. Disinfect the wound subsequently with 70% alcohol.*
- *Eye contact: Immediately flush eyes with water. Assure adequate flushing by separating the eyelids with fingers. Flush from the nose to the outer side to prevent contamination of the other eye.*
- *Mucous membrane contact: Immediately flush with water.*
- *Skin contact: Wash-off with a gauze soaked in a 0.5% chlorohexidine in 70% alcohol solution and subsequently wash with water.*
- *In case of accidental exposure the company physician (CHDR) will immediately be contacted.*
- *The CHDR physician will register all available references of the source and contact the functionary of safety, health and environment (VGM) of CHDR as soon as possible.*

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

   See 1 above; All materials used in the spill clean-up will be discarded as clinical waste, UN3291, and will be incinerated.

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

   The clinical study will be put on hold and may be discontinued in case of an undesirable effect related to the RSVΔG or G-RSVΔG that causes significant harm to human health.

References:

Widjojoatmodjo, M. N., J. Boes, M. van Bers, Y. van Remmerden, P. J. Roholl and W. Luytjes (2010). "A highly attenuated recombinant human respiratory syncytial virus lacking the G protein induces long-lasting protection in cotton rats." Virol J **7**: 114.