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	***Sweden***
(b)	Notification number	B/SE/10/EU-2010-019936-11
(c)	Date of acknowledgement of notification	2010/04/26
(d)	Title of the project	***A phase 1, single centre, dose-escalating, placebo-controlled study of a genetically modified B. pertussis strain given as a single intranasal dose to healthy adult male volunteers***
(e)	Proposed period of release	***From 2010-08-01 to 2011-06-30***

2. Notifier

Name of institution or company: ***Inserm (sponsor); Karolinska Trial Alliance, Karolinska University Hospital (Clinical investigator) Swedish Institute for Infectious Disease Control (Coordinating institution)***

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class …

(b) Identity of the GMO (genus and species)
Genetically attenuated strain of Bordetella pertussis (BPZE1)

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

Stable
(Genetic stability of BPZE1 after 20 and 27 weeks of continuous passaging in vitro and in vivo, respectively, has been demonstrated (See Feunou-Feunou, P et al. 2008. Vaccine)).

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

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

If yes, insert the country code(s) …

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

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

If yes:

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

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

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

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

If yes:

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

7. Summary of the potential environmental impact of the release of the GMOs.
The GMO is an attenuated strain of Bordetella pertussis named BPZE1. It was engineered by eliminating or genetically detoxified three B. pertussis toxins, pertussis toxin, dermonecrotic toxin and tracheal cytotoxin (See Mielcarek et al., 2006. PLoS Pathogens). Preclinical data attesting the safety of BPZE1 have been obtained in mice (See Skerry et al. 2009. Clin. Vaccine Immunology) and have contributed to down-grading BPZE1 from a biosafety level 2 organism to biosafety level 1 in France (Appendix).
The GMO is not invasive and has no selective advantage in the environment. The potential for exchange of genetic material is virtually inexistant, since B. pertussis does not harbor plasmids or conjugative transposons. In addition, B. pertussis Tohama I (background used for the BPZE1 GMO) does not harbor intact prophage genomes and is therefore incapable of producing functional phage particles.

The GMO will be administered nasally via tuberculin syringes to healthy adult male volunteers. The volunteers will stay at the study centre for 6 hours after administration of the GMO. The attenuated BPZE1 bacteria are expected to colonize the upper respiratory tract similarly to the wild-type B. pertussis. Shedding of live organism will be followed in nasopharyngeal washings performed at various intervals from the day of administration until the end of the study (6 months later). Chronic carriage of B. pertussis has not been reported and is therefore not expected.

In case of transmission to other humans, accidentally exposed, an efficient treatment against B. pertussis is commercially available and is based on administration of erythromycin. BPZE1 has been shown to be sensitive to erythromycin.
In summary, the risk assessment for this study shows a very low risk for potential environmental impact associated with administering the GMO to volunteers.

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

other, specify …

2. Name

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

3. Geographical distribution of the organism

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

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

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

Atlantic	***X***
Mediteranean	***X***

	Boreal	***X***
	Alpine	***X***
	Continental	***X***
	Macaronesian	***X***
(ii)	No	(.)
(iii)	Not known	(.)

(c) Is it frequently used in the country where the notification is made?
Yes (***in laboratories***) 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	…***B. pertussis is a human pathogen colonizing the upper respiratory tract. Mice can be infected using a high dose of microorganisms.***

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

5. (a) Detection techniques
Culture of nasopharyngeal washings on plate Bordet Gengou + 20% sheep blood . Incubation at 37°C for at least 3 days.
Molecular techniques based on PCR amplification of 16SRNA.

(b) Identification techniques
Molecular techniques based on PCR amplification of 16SRNA.

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
B. pertussis is classified as a BSL2 microorganism.

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: ***B. pertussis produces several virulence factors including toxins. Among them, pertussis toxin has been shown to induce systemic toxic effects. Tracheal cytotoxin is likely to be responsible for the cough syndrome.***

(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

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
In optimal laboratory culture conditions, the generation time is approx. 4 hours

(b) Generation time in the ecosystem where the release will take place:
Not known

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

(c) Factors affecting reproduction:
N/A

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:
Nutrients in the culture medium, temperature (37°C). The survival time in Phosphate buffer at room temperature is 18 hours.

10. (a) Ways of dissemination
Aerosol

(b) Factors affecting dissemination
Coughing of infected humans

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)
…, B/../../…
No previous genetic modifications of the recipient or parental organism has already been notified for release in the country where the notification is made.

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	(***X***)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification
Briefly, the genetic modification alter or remove three B. pertussis toxins, pertussis toxin (PTX), tracheal cytotoxin (TCT) and dermonecrotic toxin (DNT). This strain, named BPZE1, consequently expresses an enzymatically inactive PTX by altering two key amino acids for the enzymatic activity of the toxin (mutations R9K and E129G; either one of these mutations abolishes toxin activity), shows a 100 fold reduction in TCT activity by the replacement of the B. pertussis ampG gene by that of E. coli and does not produce DNT by the deletion of its structural gene.

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

(i)	transformation	(***plasmid vector was used for transformation but no plasmid material is left in the final strain***)
(ii)	microinjection	(.)
(iii)	microencapsulation	(.)
(iv)	macroinjection	(.)
(v)	other, specify …	

6. Composition of the insert

(a) Composition of the insert
The insert consists of the ampG gene from E. coli K12

(b) Source of each constituent part of the insert
The insert is of bacterial origin

(c) Intended function of each constituent part of the insert in the GMO
The insert encodes a functional AmpG transporter protein. B. pertussis AmpG is inefficient in the internalization of peptidoglycan breakdown products, such as the tracheal cytotoxin (TCT). The B. pertussis ampG gene was therefore replaced by E. coli ampG. The resulting strain expressed less than 1% residual TCT activity (background activity).

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

other, specify …

2. Complete name

(i)	order and/or higher taxon (for animals)	***Bacteria***
(ii)	family name for plants	…
(iii)	genus	***Escherichia***
(iv)	species	***E. coli***
(v)	subspecies	…
(vi)	strain	***K12***
(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:

(b) 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 (***X***) No (.) Not known (.)
Specify ***The GMO grows slightly less well in the respiratory tract of adult mice.***

(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 …

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (***X***) No (.) Not known (.)
Specify ***Since background levels of tracheal cytotoxin (TCT) are produced by the GMO and since TCT is likely to be responsible for the cough syndrome, the GMO is expected to disseminate much less efficiently than the recipient 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 GMO is strongly attenuated, does not induce airway inflammation and, in fact, protects against airway inflammation induced by allergens or viral infections.***

2. Genetic stability of the genetically modified organism
Genetic stability of BPZE1 after 20 and 27 weeks of continuous passaging in vitro and in vivo, respectively, has been demonstrated (See Feunou-Feunou, P et al. 2008. 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 (.)
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

Culture on plate Bordet Gengou + 20% sheep blood. Incubation at 37°C for at least 3 days. Silver coloured uniform colonies of B. pertussis are surrounded by a halo ring. Molecular techniques based on PCR amplification of 16SRNA.

(b) Techniques used to identify the GMO

GMO is identified by genetic characterization using PCR and sequencing techniques.

PCR analysis of the ampG and dnt loci of BPZE1: Genomic DNA is extracted from isolated colonies and used as template for the PCR using appropriate sense and anti-sense oligonucleotides. The amplified products were analyzed by electrophoresis within a 1% agarose gel in TAE buffer containing ethidium bromide and visualized under UV light.

Sequence analysis of the ptx locus of BPZE1: The DNA fragments containing the region encompassing the R9K and the E129G mutations of the ptxS1 gene are amplified by PCR from bacterial genomic DNA, using appropriate primers. The amplified DNA fragments are then directly sequenced in both directions by automated sequencing.

F. Information relating to the release

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

This application covers "a phase 1, single centre, dose-escalating, placebo-controlled study of a genetically modified B. pertussis strain (BPZE1) given as a single intranasal dose to healthy adult male volunteers" conducted in Sweden.

The proposed live BPZE1 vaccine is expected to induce protection against whooping-cough. Although all age groups are susceptible, it is most severe in infants too young to be protected by currently available vaccines. Infected adults also constitute an important reservoir for transmission of the disease to very young children. BPZE1 vaccination is expected to protect quickly after a single nasal dose. As a prerequisite for application in infants, first-in-man studies (phase I safety trials) have to be conducted in adult volunteers.

The GMO BPZE1 is formulated in solution (0.1 mL in cryovial) for nasal administration by drops. The GMO will be administered nasally via tuberculin syringes to healthy adult male volunteers. The volunteers will stay at the study center for 6 hours after administration of the GMO. The attenuated BPZE1 bacteria are expected to colonize the upper respiratory tract.

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):
Clinical Trial Alliance, Karolinska University Hospital, Stockholm

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

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
None

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
None

4. Method and amount of release

(a) Quantities of GMOs to be released:
A total of 36 volunteers (12 in each group of 3 different escalating doses) will receive the GMO BPZE1 during the study. The volunteers will enter the study dose groups in sequential order.

Group 1: 12 individuals will be vaccinated once intranasally with 1,000 colony forming units (cfu) of the organism. 4 individuals will get placebo (the diluent alone).

Group 2: 12 individuals will be vaccinated once intranasally with 100,000 colony forming units (cfu) of the organism. 4 individuals will get placebo (the diluent alone).

Group 3: 12 individuals will be vaccinated once intranasally with 10,000,000 colony forming units (cfu) of the organism. 4 individuals will get placebo (the diluent alone).

(b) Duration of the operation:
Recruitment of the first volunteers is expected to start in August 2010. The trial should start after the pollen season. Each volunteer will be followed during 6 months after administration of BPZE1. The last blood sample is expected in June 2011 and the clinical study report on Novemberr 2011.

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

Standard Operating Protocols (SOPs) are available to store, transport and administer the GMO BPZE1. All these protocols contain the appropriate measures to avoid spread of the GMO in the environment.

BPZE1 is formulated in solution. The primary packaging is a sterile 1.8 mL polypropylene CryoTube (CE marked medical device) (Nunc, Denmark). The vial closure system is a polypropylene screw cap. At the clinical trial centre (Karolinska Trial Alliance) at

Karolinska Univeristy Hospital, the GMO will be administered nasally via tuberculin syringes to healthy adult male volunteers. The volunteers will stay at the study center for 6 hours after administration of the GMO.

5. Short description of average environmental conditions (weather, temperature, etc.)
The weather in Stockholm is characterized by four well defined seasons, including at least one month with average temperatures below zero centigrade.

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.
N/A

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

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
Volunteers will receive the GMO nasally. The attenuated BPZE1 bacteria are expected to colonize the upper respiratory tract of the volunteers for a limited amount of time and to induce a potent T- and B-cell immune responses to B. pertussis antigens.

3. Any other potentially significant interactions with other organisms in the environment
None

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (***X***) Not known (.)
Give details
…

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

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

(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 potential for exchange of genetic material is virtually inexistant, since B. pertussis does not harbor plasmids or conjugative transposons. In addition, B. pertussis Tohama I (background used for the BPZE1 GMO) does not harbor intact prophage genomes and is therefore incapable of producing functional phage particles.

(b) from other organisms to the GMO:
None

(c) likely consequences of gene transfer:
None

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
Information not available

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
No potential interactions with biogeochemical processes have been identified.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Should it become indicated, the BPZE1 GMO can be identified using PCR and sequencing methods.

2. Methods for monitoring ecosystem effects
Not planned

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

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

5. Duration of the monitoring
N/A

6. Frequency of the monitoring
N/A

I. Information on post-release and waste treatment

1. Post-release treatment of the site
The clinical trial center will disinfect equipment and surface according to standard medical procedures. All materials used for nasal administration will be put in special containers and destroyed according to procedures for hospital waste. Volunteers will stay at the study center for 6 hours after nasal administration of the GMO.

2. Post-release treatment of the GMOs
All empty vials will be destroyed at the investigational site according to the standard procedures. Unusable study vaccines, i.e. expired vaccines and vaccines having experienced a cold chain break must not be administered and will be returned to Innogenetics, the company producing the GMO under GMP conditions, according to the regulations meant for the transport of GMOs.

Following verification of vaccine accountability, all unused and unusable study vaccines will be returned to Innogenetics.

3. (a) Type and amount of waste generated
1.8 mL polypropylene CryoTube , syringes, gloves, disposable aprons

3. (b) Treatment of waste
Destruction according to procedures for hospital waste

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
Unexpected spread resulting from accidental spills during any of the administration procedures will be cleaned using 70% alcohol and absorbent material. All materials used during the cleaning procedures will be destructed according to procedures meant for hospital waste.
In case of the higly unlikely transmission to other humans, accidentally exposed, an efficient treatment against B. pertussis is commercially available and is based on administration of erythromycin. BPZE1 has been shown to be sensitive to erythromycin.

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

Unexpected spread resulting from accidental spills during any of the administration procedures will be cleaned using 70% alcohol and absorbent material. All materials used during the cleaning procedures will be destructed according to procedures meant for hospital waste.

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

4. Plans for protecting human health and the environment in the event of an undesirable effect
BPZE1 GMO is a highly attenuated bacterium which has been down-graded from a biosafety level 2 organism to biosafety level 1 in France (Annex). In case of highly unlikely undesirable effect, efficient antibiotics (erythromycin) treatment can be administered to people potentially affected. There is no indication of possible undesirable effects on the environment.