

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/09/EU 2009-011478-13*  
(c) Date of acknowledgement of notification *2009/09/09*  
(d) Title of the project:  
*“A phase I trial to assess the safety and feasibility of administering plasmid DNA carrying multiple HIV-1 genes together with dermal electroporation, HIVIS 07”*

- (e) Proposed period of release *From 2009-12-01 to 2012-12-01*

2. Notifier

Name of institution or company: *Swedish institute for Infectious Disease Control, Stockholm, Sweden*

3. GMO characterization

- (a) Indicate whether the GMO is a:

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

specify phylum, class Poxviridae

- (b) Identity of the GMO (genus and species):  
*Genus: orthopoxvirus, Species: Modified Vaccinia Ankara Chang Mai Double Recombinant (MVA-CMDR)*

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

**Stable**

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 (X) No (.)

If yes:

- Member State of notification

**Sweden**

- Notification number

B/SE/05/151:2005/30190

**Our previous clinical trial in Stockholm**

**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

...

- Notification number

B/./.../...

**(Released for clinical trial in the USA, Thailand and Tanzania)**

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

**None**

**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 (X)

bacterium (.)

fungus (.)

animal

- mammals (.)

- insect (.)

- fish (.)

- other animal (.)

(specify phylum, class) *Poxvirus*

other, specify ...

2. Name

- |       |                                         |                                      |
|-------|-----------------------------------------|--------------------------------------|
| (i)   | order and/or higher taxon (for animals) | <i>Poxviridae</i>                    |
| (ii)  | genus                                   | <i>Orthopoxvirus</i>                 |
| (iii) | species                                 | <i>Vacciniavirus (cowpox)</i>        |
| (iv)  | subspecies                              | ...                                  |
| (v)   | strain                                  | <i>Modified Vaccina virus Ankara</i> |
| (vi)  | pathovar (biotype, ecotype, race, etc.) | ...                                  |
| (vii) | common name                             | <i>MVA, Modified Vaccina Ankara</i>  |

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 ( )

- (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      | .. |
| Mediterranean | .. |
| Boreal        | .. |
| Alpine        | .. |
| Continental   | .. |
| Macaronesian  | .. |

- (ii) No (X)

- (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 (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                              |     |

*No natural host known, current virus derived from cowpox and adapted to chicken embryo fibroblasts by loss of 14kb of its genome (Mayr A et al. Infection 1975, 3: 6-14). The virus does not exist in nature as it is replication-defective in the majority of cell types.*

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

N/A

5. (a) Detection techniques  
*Culture on chicken embryo fibroblasts*

(b) Identification techniques  
*Polymerase chain reaction (PCR)*

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

*Related to Vaccine against smallpox (BSL 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 (.)

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:

Not applicable as it does not replicate in nature

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

Not applicable as it does not replicate in nature

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

(c) Factors affecting reproduction:

*MVA was derived from the vaccinia strain Ankara by more than 570 serial passages in primary chick embryo fibroblasts, which severely compromised its capacity to replicate in mammalian cells (Mayr et al, 1975; 1998).*

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:

...

10. (a) Ways of dissemination

...

(b) Factors affecting dissemination

***MVA is incapable of replication in humans and thus there should be no virus shedding from the vaccinated individuals. The genetically modified viral vaccine is not able to survive, disseminate in and/or displace other organisms and is not pathogenic to animals or plants. MVA has an excellent safety profile and is non-pathogenic also in immunocompromised individuals (Im, J. et al., Expert Rev Vaccines. 2004 Aug;3(4 Suppl):S89-97.)***

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/././...

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

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

***Genes from HIV-1 inserted in order to achieve expression of HIV proteins in vivo as antigens for immunization***

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

Yes (X) No (.)

If no, go straight to question 5.

- (b) If yes, is the vector wholly or partially present in the modified organism?  
Yes (X) No (.)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

plasmid (X)  
bacteriophage (.)  
virus (.)  
cosmid (.)  
transposable element (.)  
other, specify ...

(b) Identity of the vector

*pLW17CM235envt(#19); pLW9CM240gagpol*

(c) Host range of the vector

*E. coli*

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

Yes (.) No (X)

*Nucleotide remnants from the plasmid may be present in the MVA construct, but are difficult to discriminate from the MVA sequence. There is no antibiotic resistance gene.*

antibiotic resistance (.)

other, specify ...

Indication of which antibiotic resistance gene is inserted

...

(e) Constituent fragments of the vector

*pLW17*

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

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

*transfection of CEF. The genes of interest are recombined into deletion II of MVA by the H5 promoter in the plasmid pLW17.*

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

***The pLW17CM235envt(#19) plasmid contains the truncated envelope from HIV-1 isolated in Chang Mai of CRF A\_E; The pLW9CM240gagpol plasmid contains the gag/pol sequence from an isolate from the same individual with an inactivated reverse transcriptase and a deletion of the integrase gene.***

***CEF cells were infected with MVA and then transfected with the pLW9CM240gagpol plasmid and lysed. The lysate was used to infect CEF cells. Recombinant virus plaques were identified by immunostaining against gag/pol and expanded in CEF cells. CEF cells were again infected with this recombinant and transfected with pLW-CM235env(#19) and recombinants expressing env were detected by immunostaining. This double recombinant was further plaque purified 6 times***

(b) Source of each constituent part of the insert  
***Synthetic plasmid, pLW17***

(c) Intended function of each constituent part of the insert in the GMO  
***Immunization against HIV-1 proteins***

(d) Location of the insert in the host organism

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

***Inserted in the MVA genome***

(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**

***Human Immunodeficiency Virus –1 circulating recombinant form A\_E (HIV-1 CRF A\_E)***

1. Indicate whether it is a:

- viroid (.)
- RNA virus (X)
- DNA virus (.)

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

2. Complete name

(i) order and/or higher taxon (for animals) ...  
 (ii) family name for plants ...  
 (iii) genus Lentivirus  
 (iv) species Human immunodeficiency virus type 1  
 (v) subspecies ...  
 (vi) strain CM235, CM240  
 (vii) cultivar/breeding line ...  
 (viii) pathovar ...  
 (ix) common name ...

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

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

If yes, specify the following:

(b) to which of the following organisms:

humans (X)  
 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 (X) No (.)

If yes, specify

**Human immunodeficiency virus: Risk class BSL 3**

5. Do the donor and recipient organism exchange genetic material naturally?



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

**E. Information relating to the genetically modified organism**

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?

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

Specify ...

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

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

Specify ...

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

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

Specify ...

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

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

Specify ...

2. Genetic stability of the genetically modified organism

***Stable after 2 years at -80 and 24 h at room temp.***

***The gag and env genes are expressed in 99% of the plaques after 26 passages.***

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 recombinant virus may be detected using a "nested" PCR.***

(b) Techniques used to identify the GMO  
***The identity of the MVA virus may be checked using PCR probes specific for both MVA genes and the gag, pol and env inserted genes.***

**F. Information relating to the release**

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

***Phase 1 clinical trial.***

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 (X) No (.)

If yes, specify: ***MVA has no natural host***

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):  
Venhälsan, Södersjukhuset, Karolinska University Hospital

(b) Size of the site (m<sup>2</sup>): N/A ... m<sup>2</sup>  
(i) actual release site (m<sup>2</sup>): ... m<sup>2</sup>  
(ii) wider release site (m<sup>2</sup>): ... m<sup>2</sup>

(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:  
***30 volunteers will receive two injections of 10<sup>8</sup> plaque forming units (pfu) intramuscularly***

(b) Duration of the operation:  
***Enrollment will be over 12 months***

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release  
***Protocols are available to: store, transport and administer the vaccine. Additional protocols to adequately destruct all materials that have been in contact with the***

***GMO are available. All these protocols contain the appropriate measures to avoid spread of the GMO in the environment.***

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.  
***MVA is incapable of replication in humans and thus there should be no virus shedding from the vaccinated individuals. The genetically modified viral vaccine is not able to survive, disseminate in and/or displace other organisms and is not pathogenic to animals or plants. We have so far evaluated the MVA-CMDR in one phase 1 trial in Stockholm and one phase 1/2 trial in Tanzania without detectable environmental impact.***

**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) N/A
  - (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)  
***The MVA will infect cells of the vaccine recipient and the recombinant genes will be expressed, where after the virus is cleared from the body. The infection is a single round infection, meaning that there will be no virus propagation and dissemination.***
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 *N/A*

- (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*
- (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.):

*Vaccine trials with this MVA (Sweden, USA, Thailand and Tanzania) or similar MVA constructs (USA, UK, Germany, Holland, Kenya and Uganda) have been performed in without detectable environmental impact.*

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)  
*None*

## **H. Information relating to monitoring**

1. Methods for monitoring the GMOs

*Should it become indicated the vaccine can be detected by PCR.*

2. Methods for monitoring ecosystem effects

*MVA is incapable of replication in humans and thus there should be no virus shedding from the vaccinated individuals. The genetically modified viral vaccine is not able to survive, disseminate in and/or displace other organisms and is not pathogenic to animals or plants.*

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<sup>2</sup>)

N/A

5. Duration of the monitoring

N/A

6. Frequency of the monitoring

...

#### **I. Information on post-release and waste treatment**

1. Post-release treatment of the site

*Surfaces that have been used during vaccination will be cleaned using 0.1% chloride-solution (or the equivalent). All materials used during vaccination will be put in special containers and destroyed according to procedures for hospital waste.*

2. Post-release treatment of the GMOs

*Used vaccine bottles and unused syringes will be transported back to the pharmacy at Södersjukhuset (where the vaccines are stored) using closed, unbreakable leakage-free containers. At the end of the study, all empty vaccine bottles will be destroyed according to procedures for hospital waste. Unused vaccine will be returned to the sponsor, Swedish institute for Infectious Diseases Control, according to regulations meant for the transport of GMO's.*

3. (a) Type and amount of waste generated

*Needles, syringes, cotton balls, dry adhesives, 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

*Blood and vaccine that has been spilled during any of the vaccination procedures will be cleaned using absorbent material and 70% alcohol. All materials used during the cleaning procedures will be destructed according to procedures meant for the destruction of hospital waste.*

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

*Blood and vaccine that has been spilled during any of the vaccination procedures will be cleaned using absorbent material and 70% alcohol. All materials used during the cleaning procedures will be destructed according to procedures meant for the destruction of 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  
*N/A*