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: Netherlands
(b) Notification number: B/NL/15/010
(c) Date of acknowledgement of notification: 30/11/2015
(d) Title of the project
**Safety and protective efficacy of genetically modified *Plasmodium berghei (Pb(PfCS@UIS4))* malaria parasites in healthy volunteers**
(e) Proposed period of release: From 01/03/2015 until 01/03/2020

2. Notifier

Name of institution or company: Radboud university medical center

3. GMO characterization

(a) Indicate whether the GMO is a:

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

specify phylum, class: Apicomplexa protozoan malaria parasite

(b) Identity of the GMO (genus and species)

*Plasmodium berghei*

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

There are no reports of naturally occurring exchanges of genetic material between *Plasmodium* and other organisms, including their natural hosts. Likewise, there are no reports of self-transmissible elements, mobilisable plasmids or transposons ever identified in *Plasmodium* parasites in general, or in *Plasmodium berghei* in particular.

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.

*Plasmodium berghei* is a rodent-infective *Plasmodium* species that is **not pathogenic** to humans. It is the most widely used model malaria parasite species in research laboratories worldwide. In the laboratory the natural hosts have been replaced by a number of commercially available laboratory mouse strains, and various mosquito species, including *Anopheles stephensi*, which is easily reared and maintained under defined laboratory conditions.
Wild-type (WT) *P. berghei* is stored and used under BSL1 or ABSL2 containment conditions. We have further demonstrated, in the pre-clinical studies, its safety in humans by using a variety of laboratory models, including a blood-humanized mouse model to show that it is unable to develop in human erythrocytes.
Transgenic *P. berghei* parasites have been used in laboratories for many years and pose no greater environmental risks than the non-pathogenic, wild-type, parental strain. The life cycle of the *Pb(PfCS@UIS4)* parasite is similar to the one of the parental *P. berghei* strain. The genetically modified *Pb(PfCS@UIS4)* parasite is expected to behave in a physiologically similar manner to the parental *P. berghei* strain and, like the latter, to be non-pathogenic to humans. The genetic modification introduced (insertion of the *Plasmodium falciparum* circumsporozoite protein gene in the 230p neutral locus of *P. berghei*, under the control of the *P. berghei* UIS4 promoter) is not expected to bear any influence on the non-pathogenicity of

the resulting genetically modified parasite. Firstly, the circumsporozoite protein is normally not expressed in the potentially pathogenic *Plasmodium* blood stages and plays no role in the invasion of erythrocytes by these parasites; secondly, the gene was inserted under the control of the *P. berghei* UIS4 promoter, which is only active during the asymptomatic hepatic stages of the parasite's life cycle; thirdly, the behaviour of *Pb(PfCS@UIS4)* parasite towards human red blood cells was evaluated using a blood-humanized mouse model and, like the parental *P. berghei* strain, the *Pb(PfCS@UIS4)* parasite was found to be unable to develop inside human red blood cells.
The *Pb(PfCS@UIS4)* parasite is also expected to behave similarly to its wild-type counterpart in the mosquito vector. The parasite infects the mosquito when the latter bites on an infected rodent. The parasite then undergoes a sporogonic development phase in the mosquito midgut that culminates in the invasion of the mosquito salivary glands by sporozoites. These will persist in the vector throughout the duration of the mosquito's lifespan, i.e., approximately 2 months. In the rodent host, the parasite exists in the liver for approximately 48 hours prior to initiating the blood stage of infection, which will persist until effectively treated. No latent forms of the parasite exist at any point of its life cycle.
No interactions between the *Pb(PfCS@UIS4)* parasite and other microorganisms have been described.

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

1. Recipient or parental organism characterization:

(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~~ | Parasite |
| | (specify phylum, class) | … |
| | other, specify | Apicomplexa protozoan malaria parasite |

2. Name

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

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 (X)

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

| | |
|---|---|
| Atlantic | .. |
| Mediteranean | .. |
| Boreal | .. |
| Alpine | .. |
| Continental | .. |
| Macaronesian | .. |
| Other | Africa |

(ii) No (.)
(iii) Not known (.)

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

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

4. Natural habitat of the organism

(a) If the organism is a microorganism

| | |
|---|---|
| water | (.) |
| soil, free-living | (.) |
| soil in association with plant-root systems | (.) |
| in association with plant leaf/stem systems | (.) |
| other, specify | in association with its mosquito or rodent hosts |

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

5. (a) Detection techniques

When in the liver stage of its life cycle, the organism can be detected by PCR or by immunofluorescence microscopy techniques. When in the blood stage of its life cycle, the organism can be detected by PCR or by microscopy techniques.

(b) Identification techniques
The organism can be identified by PCR techniques.

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

The recipient organism (*Plasmodium berghei*) is classified as risk group 2 according to legislation 2000/54/EG.

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

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

If yes:

(a) to which of the following organisms:

| | |
|---|---|
| humans | (.) |
| animals | (X) |
| plants | (.) |
| other | (.) |

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

*P. berghei* infections can induce morbidity and lethality in rodents.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

21 days

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

None

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

(c) Factors affecting reproduction:

At no stage of their life cycle can *Plasmodium berghei* parasites be airborne nor can they be transmitted via contact between two vertebrate or between two invertebrate hosts.
*Plasmodium berghei* **sporozoites** can be collected from mosquito salivary glands but, once isolated from the mosquito, they are only viable for a few hours. Previously, a study has shown that *Plasmodium berghei* sporozoites can also be obtained *in vitro* under very specific culture conditions (Al-Olayan, Beetsma et al. 2002; Hurd, Al-Olayan et al. 2003; Carter, Nacer et al. 2007). However follow up studies to confirm these results have not yet been performed.
*Plasmodium berghei* **merozoites** cannot survive or replicate outside their rodent host cells. They can be maintained in *in vitro* cultures in the laboratory only for the duration of one intra-erythrocytic cycle, as they cannot rupture and re-invade erythrocytes *in vitro*. *Plasmodium berghei*-infected rodent erythrocytes can be frozen and maintained at -80ºC or in liquid nitrogen. *Plasmodium berghei* blood stages can only initiate a new infection through a blood transfusion.
*Plasmodium berghei* **gametocytes** cannot survive outside the rodent or the mosquito hosts.

They can originate sporozoites in mosquitoes or under specific *in vitro* conditions.
*Plasmodium berghei* is **not pathogenic** to humans and is unable to develop or to produce gametocytes in human erythrocytes.

9. Survivability

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

| | | |
|---|---|---|
| (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:

*Plasmodium berghei* **sporozoites** can be collected from mosquito salivary glands but, once isolated from the mosquito, they are only viable for a few hours.

*Plasmodium berghei* **merozoites** cannot survive or replicate outside their rodent host cells. They can be maintained in *in vitro* cultures in the laboratory only for the duration of one intra-erythrocytic cycle, as they cannot rupture and re-invade erythrocytes *in vitro*.

*Plasmodium berghei* **gametocytes** cannot survive outside the rodent or the mosquito hosts. They can originate sporozoites in mosquitoes or under specific *in vitro* conditions.

10. (a) Ways of dissemination

The organism can only be naturally transmitted through the bite of infected vectors (female *Anopheles* mosquitoes). Mosquitoes can only be infected by biting on an infected rodent host at the specific time when parasite gametocytes are in circulation. *Plasmodium berghei* blood stages can only initiate a new infection through a blood transfusion.

(b) Factors affecting dissemination

See 8c and 9b

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

To express the circumsporozoite protein of a related *Plasmodium* species (*P. falciparum*) with the intent of eliciting immune responses against the latter.

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 | (.) |
| (ii) | microinjection | (.) |
| (iii) | microencapsulation | (.) |
| (iv) | macroinjection | (.) |
| (v) | other, specify | double cross-over recombination |

6. Composition of the insert

(a) Composition of the insert

The gene encoding *P. falciparum* circumsporozoite (CS) protein (PF3D7_0304600) as well as the *P. berghei* UIS4 (PBANKA_050120) promoter and 3'UTR regions were cloned in the pL1988 plasmid, flanked by the 230p targeting sequences (Figure 1). Plasmid pL1988 was used to insert the cloned sequences in the 230p neutral locus of the GIMO motherline PbANKA-230p;1596cl1 (Figure 4). A negative selection process was then used to obtain the genetically modified *Pb(PfCS@UIS4)* parasite (line 2266) (Figure 2). The primers used to clone the *P. berghei* UIS4 promoter and 3' UTR regions as well as the *P. falciparum* CS gene into the pL1988 plasmid are shown in Table 1.

***Table 1***

| Gene name | Gene product | Primers |
|---|---|---|
| Promoter regions | | |
| ***uis4*** | PBANKA_050120 | TAT**CCTGCAGG**GTGATAGTGTAGATTTTTTTGTTTGAC/ ATAAGAAT**GCGGCCGC**AGACGTAATAATTATGTGCTGAAAGG |
| 3' UTR regions | | |
| ***uis4*** | PBANKA_050120 | CG**GATATC**TATAATTCATTATGAGTAGTGTAATTCAG/ GGCC**GGTACC**TTTCGCTTTAATGCTTGTCATC |
| *P. falciparum* transgene | | |
| ***PfCSP*** | PF3D7_0304600 | ATAAGAAT**GCGGCCGC**CAATTCATGATGAGAAAATTAGC/ GTGT**CACCGGCG**AGATGTGTTCTTTATCTAATTAAGG |

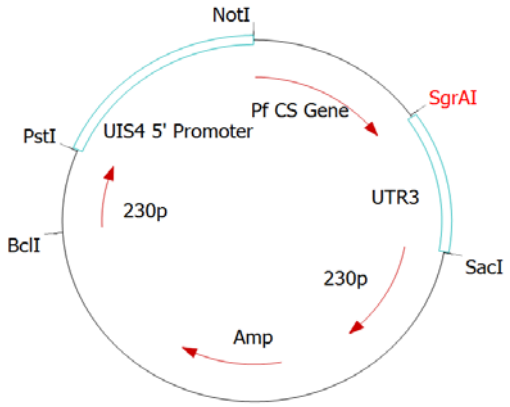


*Figure 1- The pL1988 plasmid (8067 bps)*

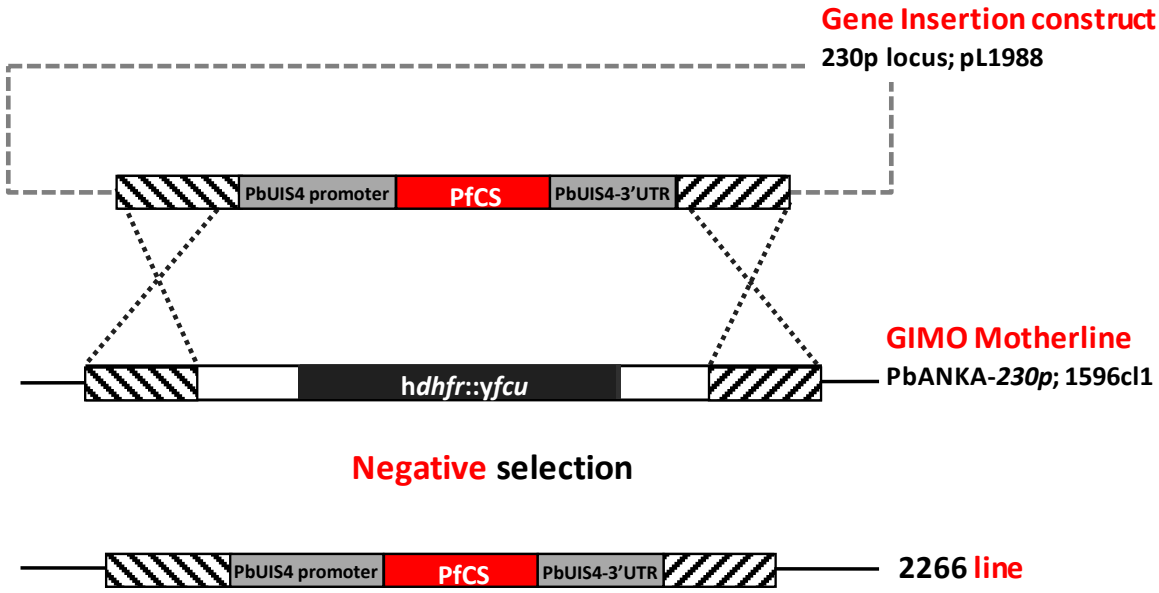


*Figure 2 - Construction of the genetically modified Pb(PfCS@UIS4) parasite (line 2266) by the GIMO method*

(b) Source of each constituent part of the insert

See (a)

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

Promoter and 3'UTR regions are intended to drive the expression of the PfCSP transgene.

(a) Location of the insert in the host organism

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

(b) 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 | (.) |
| | fungus | (.) |
| | animal | |
| - | mammals | (.) |
| - | insect | (.) |
| - | fish | (.) |
| - | other animal | (.) |
| (specify phylum, class) | | … |
| | other, specify | Apicomplexa protozan malaria parasite |

2. Complete name

| | | |
|---|---|---|
| (i) | order and/or higher taxon (for animals) | … |
| (ii) | family name for plants | … |
| (iii) | genus | *Plasmodium* |
| (iv) | species | *falciparum* |
| (v) | subspecies | … |
| (vi) | strain | NF54 |
| (vii) | cultivar/breeding line | … |
| (viii) | pathovar | … |
| (ix) | common name | *Plasmodium falciparum* |

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:

(a) 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

The donor organism (*Plasmodium falciparum*) is classified as risk group 2 according to legislation 2000/54/EG.

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 …

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

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

Specify …

(c) 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

The inserted sequence is stably integrated in a neutral locus of the recipient chromosomal DNA. Therefore, the GMO is genetically stable.

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

When in the liver stage of its life cycle, the organism can be detected by (GMO specific target genes) PCR or by immunofluorescence microscopy techniques. When in the blood stage of its life cycle, the organism can be detected by (GMO specific target genes) PCR or by microscopy techniques.

(b) Techniques used to identify the GMO

The GMO can be identified by PCR techniques with GMO specific target genes

## F. Information relating to the release

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

The GMO will be employed in Phase I/IIa clinical trials with healthy human volunteers.

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 …

Neither the recipient organism nor the GMO are naturally found in human hosts.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference): Radboudumc, Nijmegen, the Netherlands
(b) Size of the site ($m^2$): (the entire RUMC hospital), thousands of $m^2$
(i) actual release site ($m^2$): a room on the Special Malaria Unit in the Central Animal Facility: approx. 15 $m^2$, where the GMO will be administered through mosquito bites on the arms
(ii) wider release site ($m^2$): (the entire RUMC hospital), thousands of $m^2$

All exposed test-subjects are subjected to close follow-up after exposure with regular visits to the clinical trial centre. Thus, test-subjects will go home after administration.

(a) Proximity to internationally recognized 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: None

4. Method and amount of release

(a) Quantities of GMOs to be released:

75 mosquitoes infected with the GMO per volunteer

(b) Duration of the operation:

8 hours maximum

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

**Infected mosquito escape:** The mosquito- and parasite laboratories are located in separate rooms. The mosquito lab is equipped with net curtains and insect killers to prevent mosquitoes from escaping. Infected mosquitoes never leave the 'infectious-unit', which is separated from the rest of the mosquito lab by a sluice. Temperature and humidity of the climate rooms in which mosquitoes are bred and kept are continuously monitored.
To prevent cross-contamination, different parasite strains are cultured in separate rooms, mosquito-infections are performed sequentially, and mosquitoes are kept in the same cage, which is identified with a colour label, up until their use for immunization. Cages with mosquitoes infected with different strains are kept separately. To ensure that all procedures are performed appropriately, they are all performed by an experienced technician, and checked by a second technician present.

**Infected rodent escape:** The SPF mice are kept in a SPF unit in the Central Animal facility of the Radboudumc. Access is only possible for those who have received permission from the biological safety Officer (BSO) and are instructed by the responsible employee (project staff with DM II entry, cleaning staff, caretakers). Doors are always locked during proceedings. Working in the SPF-unit of the Radboudumc is strictly followed by a DM-II protocol (Containment level according to GMO regulations for genetically modified animals in association with genetically modified micro-organisms) and with a biological safety cabinet. These methods are designed to prevent genetically modified material is distributed in the environment.

**The parental species *Pb* (or other human parasites) is not present** in either the mosquito or in the human host in the Netherlands, and therefore genetic recombination between the genetic modified *Pb(PfCS@UIS4)* and wild type parasites is not possible.

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

The predominant wind direction in the Netherlands is south-west, which causes a moderate maritime climate, with cool summers and mild winters, and typically high humidity. This is especially true close to the Dutch coastline, where temperatures can be more than 10 °C (18 °F) higher (in winter) or lower (in summer) than in the (south) east of the country.

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 applicable.

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

The interactions of the GMO with the environment are similar to those of the parental organism.

1. Name of target organism (if applicable)

| | | |
|---|---|---|
| (i) | order and/or higher taxon (for animals) | … |
| (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)

The development of a malaria specific immune response.

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

Not applicable

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

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

Give details

No signs of increased competitiveness, increased invasiveness for the GMO or illness related to these have been observed in pre-clinical studies. This will be the first in-human study.

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

The absence of the natural hosts and vectors in the Netherlands makes dissemination highly unlikely.

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

*Plasmodium berghei* (*Pb*) is the wild type malaria parasite of the genetically modified *Pb(PfCS@UIS4)* and commonly used for experimental malaria infections in rodents in BSL2 laboratory. *Pb* infections can induce morbidity and lethality in rodents. The parasite cannot live freely in the environment and needs a rodent and/or mosquito host for survival.
The natural mammal host for *Pb* is the thicket rat (Grammomys surdaster), Leggada bella, Praomys jacksoni and Thamnomys surdaster living in Central Africa. *Pb* does not circulate in rodent populations in the Netherlands. *Pb* parasites can mature and proliferate in the liver and red blood cells of rodents. Malaria parasites can be transmitted from one to a next animal by direct inoculation of *Pb*-infected blood or by malaria susceptible *Pb*-infected *Anopheles stephensi*, *dureni* and *gambiae* mosquitoes. This mosquito originates from Pakistan and is kept in a BSL2 lab for over 3 decades under stringent laboratory conditions to prevent escape in the environment. *Anopheles stephensi*, *dureni* and *gambiae* are not present in natural conditions in the Netherlands.
In conclusion *Pb* is not present in wild rodents or mosquitoes in the Netherlands. Rodents in the Netherlands that hypothetically could be harmed in the highly unlikely case of a vector present in the Netherlands are described below:

| | | |
|---|---|---|
| (i) order and/or higher taxon (for animals) | | Muridea |
| (ii) | family name for plants | … |
| (iii) | genus | Apodemus, Rattus, Genus, Micromys |
| (iv) | species | Apodemus flavicollis and sylvaticus, Micromys minutes, Mus musculus, Rattus norvegicus, Rattus rattus |
| (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:

- No transfer of genetic elements of malaria parasites to other organisms have been reported.
- No transfer of genetic elements between one malaria species to another species has been reported.
- Genetic recombination/exchange between *Pb* isolates/lines can only occur after gametes which emerge from gametocytes after ingestion in a blood meal by a mosquito cross- fertilisation to form zygotes in the mosquito midgut. No exchange of genomic elements between blood-stages of different isolates have been reported in the blood of the human host or in culture.

(b) from other organisms to the GMO:

No interactions between the *Pb(PfCS@UIS4)* parasite and other microorganisms have been described.

(c) likely consequences of gene transfer:

As described in the answer to (a), the possibility of gene transfer is very unlikely. Therefore, no consequences 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.):

No references or results available.

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

Not applicable.

## H. Information relating to monitoring

1. Methods for monitoring the GMOs

The test subjects in whom the GMO will be administered, will be monitored. The GMO itself will not be monitored.
All exposed volunteers are subjected to close follow-up after exposure with regular visits to the clinical trial centre, periodic physical examinations, frequent blood sampling and recording of adverse events in a diary. After exposure, there will be a short period (35 days) of intense clinical monitoring with frequent site visits and blood examinations.
All exposed volunteers will be treated with a curative regimen of Malarone (atovaquone/proguanil) or alternatively with chloroquine or Coartem (artemether/lumefantrine), either at the time of detection of blood stage parasitemia by thick smear analysis or qPCR or 35 days after exposure to *Pb(PfCS@UIS4)* infected mosquitoes during Phase 1 (safety study). End of follow up will be 100 days after exposure to *Pb(PfCS@UIS4)*. During Phase 2 (efficacy study) exposed volunteers will be treated with a curative regimen of Malarone (atovaquone/proguanil) or alternatively with chloroquine or Coartem (artemether/lumefantrine), at the time of detection of blood stage parasitemia by thick smear during immunizations. During the challenge infection, all volunteers will receive a curative treatment, either at the time of blood stage parasitemia by qPCR or 28 post Controlled Human Malaria Infection (CHMI). End of follow up will be 100 days after the last exposure to *Pb(PfCS@UIS4)*.

2. Methods for monitoring ecosystem effects

Not applicable.

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

Whole-genome sequencing or PCR/qPCR

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

15 $m^2$

5. Duration of the monitoring

See H1

6. Frequency of the monitoring

See H1

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

1. Post-release treatment of the site

All procedures for infection of mosquitoes with *Pb(PfCS@UIS4)* and associated waste disposal are similar to procedures used for CHMI studies in Nijmegen using WT *Pf* parasite (NF54 WCB). The infected SPF mice will be killed and discarded according to the DM-II protocol (containment level according to GMO regulations for genetically modified animals in association with genetically modified micro-organisms) after the mosquitoes have been fed on the mice.

Handling of blood samples will be performed according to standard hospital procedures and licensed regulations:

- 000000 - Prevention of blood transmissible Diseases
- 025473 – Inactivation of GMO-waste and contaminated materials.
- 023832 - GMO, manufacturing and use of – and the use of ML-1 en ML-II classified facilities
- 020123 ImmGen: code of conduct

In test subjects infected with *Pb(PfCS@UIS4)* sporozoites, no asexual blood stages and gametocytes are expected to be formed since the *Pb(PfCS@UIS4)* is expected to arrest after liver stage development.

In the case of a breakthrough blood-infection: when parasites are detected in the blood, test subjects are removed from the trial, and immediately treated with Malarone (atovaquone/proguanil) or alternatively with chloroquine or Coartem (artemether/lumefantrine), resulting in removal/killing of all parasites according the Clinical Trial Protocol. Handling of infected blood samples will be performed according to standard hospital procedures and regulations.

**Waste treatment**
For waste treatment refer to the procedure of medical waste collection and treatment in the facility. Discard as medical waste in a blue SZA-bin UN 3291 and transport to the ZAVIN for immediate incineration.

The SPF mice will be killed and discarded according to the DM-II protocol (containment level according to GMO regulations for genetically modified animals in association with genetically modified micro-organisms) after the mosquitoes have been fed on the mice.

2. Post-release treatment of the GMOs

See I1. (above)

3. (a) Type and amount of waste generated

Per test-subject 5 or 75 mosquitoes will be used, killed and discarded. Furthermore disposable materials will be used for monitoring: injection syringe + needle, blood tubes, gloves.

3. (b) Treatment of waste

See I1. (above)

**J. Information on emergency response plans**

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

The procedure is designed to minimize the risk of unexpected dissemination:
separate closed room, impregnated net curtains, sluice and disposable material. In case of an escape of a malaria infected mosquito or an infected SPF mouse standard operating procedures will be followed.

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

At no stage of its life cycle the GMO can be airborne nor can it be transmitted via contact between two vertebrate or between two invertebrate hosts. The GMO cannot survive outside the rodent or the mosquito hosts.

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

The GMO cannot survive outside the rodent or the mosquito hosts.

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

In the highly unlikely case that, via unknown mechanisms, severe adverse events occur or if the GMO might cause a breakthrough infection, infected humans will be treated with Malarone (atovaquone/proguanil) or alternatively with chloroquine or Coartem (artemether/lumefantrine).