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 — United Kingdom (GB)
   - (b) Notification number — B/GB/18/R51/01
   - (c) Date of acknowledgement of notification — 14/06/2018

   (d) Title of the project
   Experimental Human Pneumococcal Challenge (EHPC) Model: **S**treptococcus Pneumoniae **N**asopharyngeal **E**xperimental carriage study of **A**ttenuated **S**trains – proof of concept in healthy adults. *Working towards vaccines for pneumonia* …

   (e) Proposed period of release — Commencing 01./09/2018 aiming to complete by 2020 but may be extended to 2021 if required due to drop out

2. Notifier
   Liverpool School of Tropical Medicine and University College London
3. GMO characterisation
   (a) Indicate whether the GMO is a:

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

   Class: Cocci
   Order: Lactobacillales

(b) Identity of the GMO (genus and species)
   Genus: *Streptococcus*
   Species: *Streptococcus pneumoniae*
   Strain: 6B (strain BHN418).

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

   High – 100% retention of antibiotic markers after multiple rounds of growth without antibiotic selection.
   Transfer of genetic material could occur to other nasopharyngeal commensals but has to our knowledge never been described, and recent data suggest that genetic exchange from

closely related streptococci such as *Streptococcus mitis* is unidirectional into *S. pneumoniae* but not vice versa (Kilian MBio. 2014 Jul 22;5(4):e01490-14. doi: 10.1128/mBio.01490-14). These data suggest there is a substantial barrier that inhibits the transfer of genetic material from *S. pneumoniae* to even closely related nasopharyngeal commensal species.

Mutant stability testing has been demonstrated using repeated culture without selection. The genetic identity of bacteria recovered from colonised individuals will be assessed using PCR on DNA extracted directly from nasal washes; using primers that anneal to the antibiotic cassette genes or the deleted genes combined with upstream or downstream sequences will detect whether revertants may have occurred with a high degree of sensitivity. Positive PCRs using deleted gene primers will demonstrate the presence of this gene in the nasopharynx; whether this is due to a revertant or co-colonisation with another *S. pneumoniae* strain will require cross referencing with culture and serotyping results and capsular gene PCR.

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 environmental impact will be restricted to humans, and is highly likely to only affect the volunteer trial subject to whom the GMO is being administered as there is a low chance of transmission to other individuals.

S. pneumoniae is only found as a human oropharynx and/or nasopharyngeal commensal; there is no environmental reservoir and *S. pneumoniae* is not a commensal of non-human mammalian species, with the exception of horses which are colonised by a subset of *S. pneumoniae* strains that do not include the 6B strain BHN418. Information relating to the recipient or parental organism from which the GMO is derived

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

1. Recipient or parental organism characterisation:

In this application, the Genetically Modified Organism (GMO) is an attenuated strain of a *Streptococcus pneumoniae* serotype 6B wild type strain (parent) that is almost genetically identical, with the only difference being deletions of two genes and their replacement with antibiotic resistance cassettes (encode resistance to spectinomycin and kanamycin). The mutations result in strains that have reduced virulence in animal models of systemic disease but are still able to colonise the nasopharynx, although this is likely to be for a shorter period of time than the wild-type parental strain.

(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	…	
(i)	…	

2. Name

Name

(i) order and/or higher taxon (for animals)	…	
(ii) genus		*Streptococcus*
(iii)species		*Streptococus pneumoniae*
(iv)subspecies	…	
(v) strain		capsular serotype 6B strain BHN418
(vi)pathovar (biotype, ecotype, race, etc.)	…	
common name		

3. Geographical distribution of the organism

The parent organism is a commensal of the nasopharynx of humans worldwide.

(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) (Human)

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? N/A
Yes (.) No (X)

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

4. Natural habitat of the organism

S. pneumoniae is only found as a human oropharynx and/or nasopharyngeal commensal; there is no environmental reservoir and *S. pneumoniae* is not a commensal of non-human mammalian species, with the exception of horses which are colonised by a subset of *S. pneumoniae* strains that do not include the 6B strain BHN418

(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	Human

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Not applicable

5. (a) Detection techniques
The samples will be processed using our published protocol (Gritzfeld, JOVE: https://www.jove.com/video/50115/experimental-human-pneumococcal-carriage) and will be plated on blood agar plate supplemented with gentamycin or selective antibiotic (spectinomycin/kanamycin). The latter will allow growth of the attenuated strain but not the wild type parent strain. Following overnight incubation at 37°C in 5% CO2, alpha-haemolytic, draughtsman shaped colonies will be sub-cultured for optochin sensitivity and the serotype will be tested using a latex agglutination kit (Staten Serum Institute).

(c) Identification techniques: a multiplex PCR targeting on *lytA* and the antibiotic resistant gene (or knocked-out genes) will be used as a verification step of the detection of the mutant strain.

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

S. pneumoniae is classified as a Hazard Group 2 pathogen on the ACDP Approved List of Biological Agents.

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

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

Pathogenic, infectivity, toxicity or allergenic:

***S. pneumoniae* commonly colonises the human nasopharynx.** *S. pneumoniae* does not known to be toxic, cause allergic responses or lead to activation of proviruses.
It has pathogenic potential and known to cause a wide range of human mucosal (otitis media, sinusitis, bronchitis) and rarely more invasive infections (pneumonia, septicaemia, meningitis). The estimated rate of invasive disease caused by serotype 6B strains per 100,000 colonisation events is less than 10 ie less than one serious invasive infection per 10,000 colonisation events (Sleeman et al J Infect Dis 2006). However these data reflect the situation in infants and will be orders of magnitude lower in young adults who have built up substantial adaptive immunity to *S. pneumoniae* (Wilson et al PLOS Pathogens 2017).

When actively colonised with wild-type 6B *S. pneumoniae* there is a low risk of pathogenicity, we have inoculated over 1000 human volunteers over a 9-year period (including 30 participants over 65 years of age, this age is known to be at higher risk of *S. pneumoniae* infections) with no cases of pneumococcal disease. The GM modifications are designed to markedly reduce the virulence of the parental 6B strain even further and therefore are markedly less likely to lead to infection than the wild type strain. The attenuated virulence of the GM strains has been confirmed in mice.

Transmission of *S. pneumoniae* from person-to-person: Transmission is thought to occur due to airborne respiratory droplets. Although, transmission can occur between close contacts but this seems to be a rare event between adults. The screening excludes participants or their close contacts who are at a higher risk of infection.

Epidemiological data provides evidence that transmission is influenced by overcrowding and concurrent viral respiratory tract infections. In a pilot study the

EHPC team found that when *S. pneumoniae* was put onto dry hands there was less nasal transmission following hand to nose contact (Connor, unpublished).
We have inoculated and followed up over 1000 healthy volunteers with the wild-type 6B strain in the Liverpool Experimental Human Colonisation model and have no reports of Serious Adverse Events. We recruited several married and un-married couples where one partner carried and the other partner did not whilst simultaneously participating in the programme.

***S. pneumoniae* is unable to colonise non-humans** (except for horses) or activate proviruses. There are no arthropod or animal vectors involved in *S. pneumoniae* transmission. There are no environmental reservoirs, insect or other animal vector, or other species in which *S. pneumoniae* 6B causes either colonization or active infections.

Transfer of genetic material could occur to other nasopharyngeal commensals but has to our knowledge never been described, and recent data suggest that genetic exchange from closely related streptococci such as *Streptococcus mitis* is unidirectional into *S. pneumoniae* but not vice versa (Kilian MBio. 2014 Jul 22;5(4):e01490-14. doi: 10.1128/mBio.01490-14). These data suggest there is a substantial barrier that inhibits the transfer of genetic material from *S. pneumoniae* to even closely related nasopharyngeal commensal species.

8. Information concerning reproduction

(a) Generation time in natural ecosystems: 24 hour generation

(b) Generation time in the ecosystem where the release will take place: as above
(c) Way of reproduction: Sexual .. Asexual X (binary fission)
(c) Factors affecting reproduction: nutrient supply, pH, oxygen tension, temperature within the nasopharynx

9. Survivability – not applicable
(*S. pneumoniae* can only survive for prolonged periods as a commensal of the nasopharynx is its natural habitat. Although it spreads between humans by indirect and direct contact it is thought that it can only survive in the environment and on skin surfaces for hours.)

(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	none of the above

(b) relevant factors affecting survivability: poor survivability – dies once allowed to dry out on any surface

10. (a) Ways of dissemination

Transmission of *S. pneumoniae* from person-to-person (only humans) is thought to occur due to airborne respiratory droplets. Epidemiological data provides evidence that transmission is influenced by overcrowding and concurrent viral respiratory tract infections. The EHPC team found that when pneumococcus was put onto dry hands there was less nasal transmission following hand to nose contact (see Q12 Connor, unpublished). There are no arthropod or animal vectors involved in *S. pneumoniae* transmission.

Musher, D.M., *How contagious are common respiratory tract infections?* N Engl J Med, 2003. **348**(13): p. 1256-66.

Hodges, R.G. and L.C. Mac, *Epidemic pneumococcal pneumonia; the influence of population characteristics and environment.* Am J Hyg, 1946. **44**(2): p. 193-206.

(c) Factors affecting dissemination
Close proximity between humans, active respiratory viral infections, age of potential recipient (higher transmission to children, especially infants

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/../../…
None

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
Substitution of a gene with an important role in virulence with an antibiotic resistance cassette. The GM modifications are designed to markedly reduce the virulence of the parental 6B strain even further and therefore are markedly less likely to lead to infection than the wild type strain. In future these data from inoculating healthy volunteers may contribute to vaccine development.

3. (a) Has a vector been used in the process of modification?
Yes (.) No (x)

If no, go straight to question 5.
(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (.) No (.)

If no, go straight to question 5.
4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

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

(b) Identity of the vector …
(c) Host range of the vector …

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

	Yes	No
antibiotic resistance	(.)	
other, specify	…	

Indication of which antibiotic resistance gene is inserted …

(e) Constituent fragments of the vector …

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

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

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

(i)	transformation	(x) of DNA fragments made by overlap extensions PCR
(ii)	microinjection	(.)
(iii)	microencapsulation	(.)
(iv)	macroinjection	(.)
(v)	other, specify …	

6. Composition of the insert

(a) Composition of the insert
Either kanamycin or streptomycin antibiotic resistance cassette
(b) Source of each constituent part of the insert
PCR amplification of kanamycin or streptomycin antibiotic resistance cassettes from existing resistant bacterial strains
(d) Intended function of each constituent part of the insert in the GMO
Marker to identify the GMO *S. pneumoniae* 6B strain and allow easy identification of these strains from other *S. pneumoniae*

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (x) completely replaces the target gene
- 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)	…
(ii)	family name for plants	…
(iii)	genus	*Streptococcus*
(iv)	species	*Streptococcus pneumoniae*
(v)	subspecies	…
(vi)	strain	6B BHN418…
(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: described above – *S. pneumoniae* is a commensal with pathogenic potential. The GMO strains have markedly reduced pathogenic potential compared to the wild type parental 6B strain.

(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 category UN 3373 (B)

S. pneumoniae is classified as a Hazard Group 2 pathogen on the ACDP Approved List of Biological Agents.

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

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

Specify The GMO strains are attenuated in virulence; in mouse models of infection they are unable to cause significant septicaemia and cause lower levels of infection in the lungs (about 1 $\log_{10}$ fewer bacterial CFU recovered from infected mice)

2. Genetic stability of the genetically modified organism

All four of the GMO *S. pneumoniae* strains are stable after 3 rounds of broth culture without selective pressure (each of which results in an increase in CFU by 250000% over 8 hours): 100 of 100 colonies tested retained resistance to both streptomycin and kanamycin showing a high degree of mutant stability.

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

Yes (X) No () 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 pathogenic potential of the GMO *S. pneumoniae* is considerable attenuated by the mutations, but the GMO strains probably still do retain a low pathogenic ability albeit much weaker when compared to the parental wild-type *S. pneumoniae* 6B strain.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

Initial screening visits will identify participants who have natural carriage of *S. pneumoniae* using nasal wash and throat swabs; positive samples will exclude a participant from the trial. Following the initial nasopharyngeal inoculation with GMO *S. pneumoniae* 6B on Day 0 the subjects will then be investigated for GMO colonization of their nasopharynx by nasal wash on days 2, 6, 14, 16, 22, 27 and 36. On the last visit prior to challenge day 36 all participants are prescribed antibiotics to clear or reduce carriage (Amoxicillin).

Participants are challenged with 6B *S. pneumoniae* (wild type) after 6 months then attend follow up visits that include nasal washes to identify bacteria on days 2, 7,14.

(b) Techniques used to identify the GMO

The samples will be processed using our published protocol (Gritzfeld, JOVE: https://www.jove.com/video/50115/experimental-human-pneumococcal-carriage) and will be plated on blood agar plate supplemented with gentamycin or selective antibiotic (spectinomycin/kanamycin). The latter will allow growth of the attenuated strain but not the wild type parent strain, as it is only included in the antibiotic resistance cassette used for gene replacement. Following overnight incubation at 37°C in 5% CO2, alpha-haemolytic, draughtsman shaped colonies will be sub-cultured for optochin sensitivity and the serotype will be tested using a latex agglutination kit (Staten Serum Institute). As well as culture a multiplex PCR targeting *lytA* and the antibiotic resistant genes (or knocked-out genes) will be used as a high sensitivity method to detect the presence of GMO *S. pneumoniae* strains.

F. Information relating to the release

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

This is a clinical trial to investigate whether nasopharyngeal administration of GMO *S. pneumoniae* 6B strains induces a protective immune response that prevents recolonisation

with wild-type *S. pneumoniae* 6B and therefore could potentially prevent diseases caused by *S. pneumoniae*.

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):
Deliberate release from the Accelerator Research Clinic 3rd Floor Liverpool School of Tropical Medicine Accelerator Building 3rd Floor, 1 Daulby Street, Liverpool L7 8XZ
Grid Reference: SJ 35782 90814

.

(b) Size of the site (m^2): … m^2
(i) actual release site (m^2): 10 m^2
(ii) wider release site (m^2): N/A as participants may travel in the UK

(e) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected: none
N/A

(f) 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:
Participants re inoculated with 80 000 CFU / nostril in 100µL (deliberate release)

(b) Duration of the operation:
Study duration aims to complete by 2020 with a possible extension to 2021 in the event of a higher dropout rate

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
There is no evidence of transmission between adults in previous EHPC studies inoculating with the wild type 6B from over 1000 participants who have completed follow up.

5. Short description of average environmental conditions (weather, temperature, etc.)
Not applicable

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.
The Experimental Human Pneumococcal Carriage Model has inoculated participants with the wild type 6B strain over a nine year period with over 1000 participants. The GMO has not previously been released.

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)	Lactobacillales…
(ii)	family name for plants	…
(iii)	genus	…*Streptococcus*
(iv)	species	… *Streptococcus pneumoniae*
(v)	subspecies	…
(vi)	strain	…6B BHN418
(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)

There is a theoretical risk of transfer of antibiotic resistance to other nasopharyngeal bacterial species (of which there are a large number covering a range of bacterial geni and species). This risk is low as:

(a) *S. pneumoniae* has not been shown to donate genetic material to other bacterial species during colonization of the human throat, and there is evidence that even with its closest genetic relation *S. mitis* gene transfers are unidirectional from *S. mitis* to *S. pneumoniae*, and not vice versa.

(b) Bacterial antibiotic resistance needs selective pressure to be be acquired and maintained and kanamycin and streptomycin are only used in clinical practice for very unusual clinical conditions, hence we can ensure the subjects used for this trial will exclude anyone who has any chance of receiving either antibiotic.

There is no planned interaction between the GM strains and the wild type 6B strain; each strain will be inoculated into separate groups of subjects. Although all groups will be rechallenged after 6 months with the wild type 6B strain this is after all subjects have been treated with amoxicillin to clear colonising bacteria and is a considerable length of time longer than colonisation is expected to last. In addition, before the administration of the wild type 6B strains after 6 months all subjects will be checked for persisting colonisation with S. pneumoniae.

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

Some of the gene deletions affecting virulence can also negatively affect the ability of the GMO *S. pneumoniae* strains to colonise the nasopharynx, and the others will have no effect (previously assessed in mice). Hence the GMO strains will either have reduced or a similar ability as the wild type strain to colonise the nasopharynx.

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

None. GMO *S. pneumoniae* persistence in the trial subjects nasopharynx will be terminated using antibiotics given at day 36. There is no environmental reservoir and transmission to other humans in the EHPC subjects seems to be a rare event (discussed above).

S. pneumoniae can only survive for prolonged periods as a commensal of the nasopharynx is its natural habitat. Although it spreads between humans by indirect and direct contact it is thought that it can only survive in the environment and on skin surfaces for hours, and this is supported by our unpublished data as discussed above.

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

This is largely restricted to other *S. pneumoniae* strains. Transfer of genetic material could occur to other nasopharyngeal commensals but has to our knowledge never been described, and recent data suggest that genetic exchange from closely related streptococci such as *Streptococcus mitis* is unidirectional into *S. pneumoniae* but not vice versa (Kilian MBio. 2014 Jul 22;5(4):e01490-14. doi: 10.1128/mBio.01490-14). These data suggest there is a substantial barrier that inhibits the transfer of genetic material from *S. pneumoniae* to even closely related nasopharyngeal commensal species. Natural carriers of *S. pneumoniae at the screening appointment will be excluded from the study prior to release (inoculation of the individual).*

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:
highly unlikely

(b) from other organisms to the GMO:
unlikely

(d) likely consequences of gene transfer:
…no serious issues; transfer of antibiotic resistance to another nasopharyngeal commensal is unlikely to be maintained in the absence of antibiotic pressure, and the antibiotics selected will not be used in the trial subjects. …

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.):
Not applicable

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
Not applicable

The Genetically Modified Organism (GMO) is less virulent as it is an attenuated strain of a *S. pneumoniae* serotype 6B wild type strain (parent). It is almost genetically identical, with the only difference being deletions of two genes and their replacement with antibiotic resistance cassettes (encode resistance to spectinomycin and kanamycin). The mutations result in strains that have reduced virulence in animal models of systemic disease but are still able to colonise the nasopharynx, although this is likely to be for a shorter period of time and lower density than the wild-type parental strain. This shorter duration of colonisation of the GMO will provide less opportunity for interaction with other commensal species.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
(a) Culture of nasal washes with identification of GMO *S. pneumoniae* as described above.
(b) PCR amplification of the lytA gene to identify presence of *S. pneumoniae*, and of the kanamycin and spectinomycin resistance cassettes to identify persistence of resistance elements

The GMOs that are cultured from nasal wash samples from volunteers can be easily confirmed to be the test and control strains through standard diagnostic microbiology techniques. The combination of the microbiological and molecular methods described above enables the detection and identification of both parent and attenuated strain with 100% accuracy.

2. Methods for monitoring ecosystem effects
as above

S. pneumoniae can only survive for prolonged periods as a commensal of the nasopharynx is its natural habitat. Although it spreads between humans by indirect and direct contact it is thought that it can only survive in the environment and on skin surfaces for hours, and this is supported by our unpublished data as discussed above. Shedding will be performed in a subset of subjects to assess *S. pneumoniae* persistence on skin surfaces.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
As above and PCR to amplify the resistance gene cassettes from nasal washes will be used as a sensitive screen for transfer of antibiotic resistance to other nasopharyngeal bacteria.

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

Not applicable as deliberate release

5. Duration of the monitoring
09/2018 aiming to complete by 2020 but extended to 2021 if required due to dropout

6. Frequency of the monitoring

Participants are monitored following the initial nasal inoculation to detect carriage of the GMO at six follow up visits that include a nasal wash during the first 36 days. Positive carriers are prescribed an antibiotic the bacteria are known to be sensitive to. Prior to a challenge with the wild type bacteria to assess the immunological response they attend a re-screening appointment and three further visits.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Laboratory disposal of the inoculum. After the completion of inoculation session, the remaining volume of inoculum is safely transferred to the laboratory facilities. A fraction is used on dilution plate to determine the dose received and the excess of inoculum is discarded in 1% aqueous solution of Virkon. Similarly, tips and dilution tubes are decontaminated in Virkon and all the solid waste is autoclaved as per LSTM clinical waste policy (SOP 004).

2. **Post-release treatment of the GMOs**

As this is deliberate release the participants return to the community following inoculation.

3. (a) Treatment of waste

Any waste generated in the laboratories that is classified as clinical waste is placed within specifically labelled yellow clinical waste sacks which are housed in yellow bins clearly marked “clinical waste”. The yellow sacks are then taken to the clinical waste skips. The Laboratory Manager or deputy fills in the required paperwork and affixes the correct waste type label to the skip. This procedure involves placing the yellow sack/s into a wheelie bin which is then used to transport the sacks to the clinical waste skips. The service lift should be used when transporting waste between floors. The wheelie bin is required to be cleaned thoroughly with disinfectant at least once a month, and after any spillage within. Once a week a commercial waste disposal company comes on site to collect the waste and take it for incineration. After the completion of inoculation session, the remaining volume of inoculum is safely transferred to the laboratory facilities. A faction is used on dilution plate to determine the dose received and the excess of inoculum is discarded in 1% aqueous solution of Virkon. Similarly, tips and dilution tubes are decontaminated in Virkon and all the solid waste is autoclaved as per LSTM clinical waste policy (SOP 004).

J. Information on emergency response plans

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

This risk of spillage is minimized during transit and the inoculation procedure.

- the inoculum is prepared in the dedicated research laboratory adjacent to the Accelerator Research Clinic
- a buddy system the laboratory scientist and the clinician check the inoculum and administer at the patient’s side

- A safety person is allocated to ensure there are no interruptions during the procedure and will wear a red apron in the area of inoculation to alert others.
- In the event of spillage the area will be cleaned using 70% ethanol. The staff will wear protective gloves and aprons then dispose of the waste in a clinical waste bin.

2. Methods for removal of the GMO(s) of the areas potentially affected
   Virkon may be used for decontamination.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
   Any waste generated in the laboratories that is classified as clinical waste is placed within specifically labelled yellow clinical waste sacks which are housed in yellow bins clearly marked “clinical waste” as per LSTM SOP 004.

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

   The EHPC Model with the wild type bacteria is well established and will be implemented in this clinical trial including screening, exclusion criteria and implementation of an established safety protocol. Additional measures for this study include evaluating safety after the first 5 - 10 participants and monitoring shedding on the hands of carriage positive participants.