Advisory Committee on Releases to the Environment

Advice on an application for deliberate release of a GMO for research and development purposes

Applicant: University of Southampton, Faculty of Medicine.

Application: A clinical study of a genetically modified (GM) *Neisseria lactamica* in adults to determine its safety and efficacy.

Ref: 25/R50/01

Date: August 2025

Advice of the Advisory Committee on Releases to the Environment (ACRE) under section 124 of the Environmental Protection Act 1990 to the Secretary of State for Environment, Food and Rural Affairs and Ministers of the Welsh Assembly Government.

ACRE is satisfied that the information provided by the applicant in accordance with the current regulations on the deliberate release of genetically modified organisms (GMOs), demonstrates that the 'release' of this GMO under the conditions of the study has been fully assessed and that the risks of adverse effect on human health or the environment are negligible. ACRE therefore sees no reason for the release not to proceed.

Background

In July 2025 ACRE considered an application from the University of Southampton for a clinical study involving the release of a genetically modified (GM) *Neisseria lactamica* in accordance with the Genetically Modified Organisms (Deliberate Release) Regulations 2002 (as amended). Members assessed the environmental risks (including risks to humans who have not been administered this GM bacterium) associated with the release of this GMO under the conditions of the clinical study set out in the application.

No public representations were received on this clinical study.

The wild-type organism *N. lactamica*, is a bacterium which lives only in the nose and throat of humans, where it resides as a harmless colonising commensal. This bacterium is carried most commonly by infants and toddlers and becomes less common in older children and adults. It is closely related to a similar bacterium, *Neisseria meningitidis*. The latter organism also lives in the nose and throat of humans and transmits between people in very close contact. *N. meningitidis* carriage is mostly harmless, but in a very small proportion of people who are carriers, the bacterium may enter the bloodstream and cause meningococcal disease, including meningitis.

A wild type *N. lactamica* strain was genetically modified by inserting two genes from *N. meningitidis* into its chromosome with the result that two additional proteins, Neisseria Adhesin A (NadA) and Factor H-binding protein (FHbp) are expressed. Both are proteins made naturally by *N. meningitidis*.

Because the shape of the FHbp protein varies between strains of *N. meningitidis*, it was necessary to make four different strains of genetically modified *N. lactamica*. Each of these four strains is able to make one different variant of FHbp, but all the strains make the same variant of NadA.

The aim of the experiment is to increase the degree to which *N. lactamica* "looks like" *N. meningitidis* to the immune system. The four strains will be mixed together in equal numbers before being used to infect study participants. The name of this mixture of bacterial strains is 4xrNlac.

The intention is to release the genetically modified strains of *N. lactamica* in two clinical studies, collectively titled "The GM-Nlac Study". In the pilot study the aim is to ensure that the applicant can effectively and safely induce carriage with 4xrNlac. Following this the main study will enrol up to 62 participants who will be randomised into two groups, one group will be challenged with 4xrNlac, whilst the other will receive a control inoculum that does not contain any bacteria. The aim is to establish whether, after becoming asymptomatically infected with one or more strains of genetically modified *N. lactamica*, a person becomes immune to asymptomatic carriage of the same genetically modified *N. lactamica* a second time. This is the first application for deliberate release within the UK of this GMO.

The GMO

A wild type *N. lactamica* strain was genetically modified by inserting two genes from *N. meningitidis* into its chromosome. The applicant notes that *N. lactamica* is extremely resistant to acquiring genes through horizontal gene transfer, despite the natural competence of the micro-organism. This required the applicant to develop a novel, hypermethylated DNA-based transformation system as a precursor to carrying out the genetic modification. This system works with DNA in which every deoxycytosine nucleotide residue is methylated, in the form of 5-methyl-deoxycytosine.

The resulting GM *N. lactamica* expresses the two additional proteins, Neisseria Adhesin A (NadA) and Factor H-binding protein (FHbp). Both are proteins made naturally by *N. meningitidis*. However, the shape of the FHbp protein varies between strains of *N. meningitidis*, and it was necessary to make four different strains of genetically modified *N. lactamica*. Each of these four strains is able to make one different variant of FHbp, but all the strains make the same variant of NadA.

The four strains will be mixed together in equal numbers before being used to infect study participants. The name of this mixture of bacterial strains is 4xrNlac.

ACRE was content with the extent to which the applicant had described the methodology involved in the genetic modifications carried out on the wild-type *N. lactamica,* and that the resultant GM micro-organisms were well characterised. The committee noted that expression of the *N. meningitidis* epitopes (namely NadA and

FHbp) was intended to stimulate an immune response that may contribute to immunity/resistance to *N. meningitidis* infection. One of these, NadA, has been used previously in a GM *N. lactamica* release, (see next paragraph), but this time is coupled with FHbp. Furthermore, ACRE noted and were content with the reasoning behind why each of the genes coding for FHbp were changed in a very small way before recombination into N. lactamica. This alteration was designed such that each of the FHbp proteins was no longer able to bind to Factor H and therefore unable to interfere with a normal immune response.
Although this is the first application for deliberate release within the UK of this GMO, the application makes reference to a previous controlled human infection model experiment (CHIME) study. This study was also conducted at the University of Southampton under a deliberate release consent, Defra Ref: 17/R50/01. That release similarly used GM *N. lactamica*, with strains that expressed either NadA (strain 4NB1) or which contained a gene expression cassette without a coding sequence (i.e. a wild type-equivalent, but genetically modified control) (strain 4YB2).

Phenotypic characterisation

ACRE was content with the applicant's description of the resulting phenotype of the GMO, as far as was required to adequately risk assess the clinical study with regards to adverse effects on human health and the environment. The applicant did not expect the mixture of GM *N. lactamica* strains to cause any symptoms or disease. The main difference between *N. lactamica* and *N. meningitidis* is the ability of the latter to make a protective capsule of sugars on its surface. This capsule is essentially what allows *N. meningitidis* to survive in the bloodstream and cause disease. Neither naturally occurring, nor the genetically modified strains of *N. lactamica* make such a capsule and are therefore unable to cause disease.

NadA

One of the introduced expressed proteins, NadA, helps *N. meningitidis* to stick to cells; it does not allow it to survive in the bloodstream or to invade cells. NadA, therefore, will not make the genetically modified strains of *N. lactamica* described in this application capable of causing disease. Furthermore, ACRE suggested that if the introduction of NadA increased the risk of pathogenesis, that might have been tested and observed in the previous study of GM *N lactamica* that expressed only NadA. To this end, the line used in that earlier study had been tested in laboratory studies, and did increase cell adhesion to the test cell line but not internalisation into cells. Also, as used in the study it resulted in "no reported adverse events attributable to the infection" and no colonisation of (a relatively small number of) close-living individuals. So those results together raised no concerns for the Committee.

In considering their advice, ACRE asked an additional question about the presence of the NadA. The committee noted that NadA was not present in wild-type *N. lactamica*, then was it that host immunity to the GM strains' NadA protein would also risk increasing immunity to other *N. lactamica* antigens. Because the point of the NadA is to assist in immune stimulation against *N. meningitidis*, would administering the GM strain serve to increase subsequent immune responses against both *N. lactamica*, as well as to the intended target? The competitive exclusion of *N. meningitidis* by *N. lactamica* appears to be important, as one can cause disease and the other does not.

Therefore, from a risk to human health perspective, any interventions that could reduce the persistence of *N. lactamica* could be a bad thing. That is could stimulation of an immune response to *N. lactamica* tip the balance of competitive exclusion in an unintended direction, by reducing *N. lactamica* long term persistence?

The applicant provided some additional information on the possible enhancement of the immune response to *N. lactamica*. The planned studies will be performed in adults where *N. lactamica* is carried infrequently. For example, in a recent UK-based cross-sectional survey of *N. meningitidis* carriage amongst individuals aged 15-19 years (MenCar4 survey), *N. lactamica* was only carried by 225 of 19641 participants (1.15%, 95% CI 1.00 – 1.30) (Maclennan et al, 2021). These dynamics are relatively stable, with similar findings demonstrated in previous large-scale UK meningococcal carriage surveys (MenCar 1-3, 1999-2001). This is in stark contrast to carriage studies performed in children where *N. lactamica* carriage peaks at >40% amongst 1–2-year-olds (Bennett et al, 2005).

From this observation, the applicant concluded that any detrimental effect of GM *N. lactamica* on subsequent acquisition of wild-type *N. lactamica* (above and beyond that observed following colonisation with the wild-type) was likely to have only a minimal impact on *N. lactamica* colonisation at the population level in UK adults. Furthermore, in a previous GM *N. lactamica* study results suggested that specific humoral and B cell responses were induced against the NadA expressed in the GM *N. lactamica* strain. However, the presence of NadA itself did not significantly affect the immune response magnitude to non-NadA epitopes contained within the *N. lactamica* backbone or affect the colonisation phenotype. It is currently unknown as to whether a sustained episode of colonisation with GM *N. lactamica* will provide protection against subsequent GM *N. lactamica* colonisation and this objective forms the primary outcome of the planned study.

However, the applicant agreed that ACRE had raised a relevant point for future study. That is to say, an understanding of whether GM *N. lactamica* impacts upon colonisation with commensal *Neisseria* spp. as well as *N. meningitidis* will be important in the longer term, particularly if this programme was to be expanded into children.

FHbp

When made by N. meningitidis during an infection, the FHbp proteins bind to the human complement protein, Factor H, which is a protein in blood that effectively stops the immune system from attacking the body. By binding Factor H, the bacterium therefore attempts to stop the immune system from attacking it.

Although FHbp is not as important as the capsule of sugars in protecting N. meningitidis in the bloodstream, it does contribute to the survival of the bacteria in this environment. ACRE therefore noted that, by adding FHbp to GM *N. lactamica*, there might inadvertently be an increase in risk to study participants, due to the FHbp binding to complement and thus inhibiting complement dependent killing of the bacteria. However, this has been circumvented by altering the Factor H binding site of the four FHbp epitopes and bacteria expressing these have been shown *in vitro* to be susceptible to serum mediated killing. The committee noted that there was nothing

else about FHbp that would make the GM N. lactamica strains capable of causing disease.

Further, ACRE were content that the evidence for loss of Factor H binding from each FHbp GM construct as presented in the application was clear. Evidence was presented from both culturing, where no increase in adhesion was recorded, and in test cell lines where no internalisation change was observed.
ACRE concluded that, regarding the risk of genetic transfer from *N. lactamica* to *N. meningitidis*, there was nothing about the genetic modifications that would necessarily make this more likely to happen. Furthermore, if genetic transfer to *N. meningitidis* were to happen, the modified FHbp proteins are no longer able to bind to Factor H, as described above. This would compromise the immune evasion capacity of the acceptor *N. meningitidis* strain and would most likely result in the pathogen becoming less fit. In addition to this, acquisition and stable expression of the NadA protein would likely only serve to greater align the transformed strain with the bactericidal antibody profile generated by Bexsero (a meningitis B vaccine containing NadA and FHbp). This would then offer greater protection against invasive meningococcal disease caused by the transformed strain.

ACRE also noted the applicant's comment that, given that both genes under consideration code for meningococcal outer membrane proteins, there would be approaching zero risk that such hypothetical genetic transfer events would lead to a significant gain-of-function in a receiving *N. meningitidis*, or that the transformed *N. meningitidis* strain would undergo radical shifts in ecology or lifestyle.

The Clinical Study

The intention is to release the genetically modified strains of *N. lactamica* in two clinical studies, collectively titled "The GM-Nlac Study". The applicant will administer a relatively low dose (400,000 CFU) of a mixture of 4 different genetically modified *N. lactamica* strains (4xrNlac) intranasally to healthy participants (this is known as 'challenge') and allow the bacteria to live asymptomatically in the nose and throat of these participants for a period of time.

In the first, pilot study, ten participants will be challenged with 4xrNlac, they will be followed up for a period of 28 days. Carriage with 4xrNlac will be cleared with oral antibiotics at the end of the study. This pilot study aims to ensure that the applicant can effectively and safely induce carriage with 4xrNlac. Following completion of this study, the main study will then enroll up to 62 participants who will be randomised into two groups, one group will be challenged with 4xrNlac, whilst the other will receive a control inoculum that does not contain any bacteria. Following challenge, they will be monitored for a period of 56 days, at which point all participants will receive antibiotics to clear the bacteria from carriage if they are present.

There will then be a second challenge at which time all participants will receive 4xrNlac, with a further 56-day follow-up period. After this second period all participants will be given a second dose of oral antibiotics to clear carriage at the end of the study.

During the two studies, participants will be closely monitored for carriage of 4xrNlac, and for any symptoms or illness. This monitoring will also assess an array of immune responses specifically directed against the genetically modified *N. lactamica.* The applicant will also collect samples from their noses and throats at different time points.

The overall aim of this study is to investigate whether carriage of genetically modified *N. lactamica* induces a 'protected against carriage' phenotype, in which participants who received 4xrNlac at the first challenge are then protected against carrying exactly the same strains of genetically modified *N. lactamica* after the second challenge, in comparison to those participants who received the control (bacteria-free) inoculum at the first challenge.

If the applicant observes a 'protected against carriage' phenotype in their participants, use will be made of samples taken from individuals to measure a variety of immune responses and attempt to work out what makes up a particular participant's 'fingerprint of protection'.

ACRE advised that while this will be a first in-human application of this GMO, the approach and risk assessment has been thoughtful and thorough, and was based on the previous experience with the NadA containing GMO and a biological understanding of the parent organisms. Previous experience suggested little shedding and no acquisition by bedroom sharers. In addition, the committee noted that there were mechanisms in place, based on the GMO being highly sensitive to antimicrobials used to reduce nasopharyngeal carriage and treat *N meningitidis* infections. These will be used at the end of the study period and so will reduce shedding and also could treat any (highly) unlikely infection that did occur.

Eligibility criteria

ACRE were cognisant of the fact that, although the proposed studies will be relatively small scale, some of the studies which generated data used in the proposal were even smaller. For example, the study concluding no transmission between bedroom sharers had only 9 participants. Therefore, ACRE were reassured that the current study would continue to monitor close contacts, with bedroom sharers of challenge participants enrolled as contact participants to assess for onward transmission. This will help build up the knowledge base for any future studies.

Challenge and contact participants will be enrolled according to specific eligibility criteria set out in the application, including the exclusion of those with regular occupational or household contact with children under 5 years, who are known to more commonly become colonised with wild type *N. lactamica*. However, ACRE and competent authority assessors asked if, within these criteria, prior vaccination against meningitis B would also exclude participation. This question was raised due to the introduction of the immunogenic component FHbp into the GM *N. lactamica* strains, a component that is also found in both Bexsero and Trumenba vaccines. ACRE noted the applicant's response, namely that they plan to exclude participants previously vaccinated with Bexsero/Trumenba as the impact of prior vaccination on GM-Nlac uptake is not predictable; albeit not one that would impact the environmental risk. However, the applicant noted that this exclusion is not expected to impact recruitment as meningitis B vaccination was only introduced into the UK infant vaccine schedule

in 2015.

The same eligibility criteria will involve screening for meningococcal carriers but does not explicitly say anything about excluding individuals who are currently colonised by *N. meningitidis*. The applicant subsequently clarified that participants will be screened for meningococcal carriage both at an initial screening visit, and at a pre-challenge visit approximately 5 days prior to inoculation. Those with active carriage at either of these timepoints will be excluded and so will not be inoculated with 4xrNlac. This is an important part of the study design as it is known that active *N. meningitidis* carriage may impede *N. lactamica* take-up and confound immunological analyses. However, it is possible that some participants might acquire *N. meningitidis* between the 'pre-challenge visit' and the 'inoculation visit'. This would not be detected prior to inoculation, and therefore, it is not possible to mitigate totally against the eventuality that a participant carrying *N. meningitidis* is amongst those inoculated. In addition, the applicant cannot control for new natural meningococcal acquisition events that occur throughout the study in participants colonised with GM-Nlac. Participants found to be *N. meningitidis* colonised following inoculation will not be withdrawn from the study for this reason but may be excluded from immunological analyses.

ACRE was minded that so far as routes to onward transmission are concerned, conducting a dental exam or treatment might also be an activity with a high risk of transmission. Therefore, the committee recommended that, as an addition to the eligibility criteria, participants should be required to either avoid such contact during the study or, if this is not possible, inform their dentists.

ACRE were concerned if there were the possibility that the alterations in the GM *N. lactamica* might change its ecology in humans in such a way as to make transfer of genes from it into *N. meningitidis* more likely, and whether there might be any concerns at all about the possible impact of that on pathogenicity. However, the committee appreciated that the proposed experiments should make the co-existence of the two organisms in one person less likely.

In addition, ACRE observed that the pilot approach with safety monitoring between cohorts, along with the monitoring in place with access to support, should detect any unexpected events.

Comment

Following a detailed consideration of the application, along with seeking clarification of the eligibility criteria, ACRE was content that the environmental risk assessment provided by the applicant was thorough and included sufficient consideration of the risks to human health and the environment as well as a good description of appropriate measures employed in order to minimise these risks.

ACRE noted that this study is a logical progression from the previous work of this applicant and that this GM construct can reasonably be assumed to be non-pathogenic with only humans as the host. Therefore, the GMO has little opportunity to spread or persist in the environment. Indeed, it could be argued that even if it persisted for a period of time the consequence would be negligible. Furthermore, ACRE noted that their deliberations on the previous study by the applicant concluded that it represented

negligible environmental risk, and the committee could not see anything in the current proposal that would increase the environmental risk.

References

Maclennan, J. M. et al. (2021) Meningococcal carriage in periods of high and low invasive meningococcal disease in the UK: comparison of UKMenCar1-4 cross-sectional survey results. *The Lancet Infectious Diseases* **21**: 677-687.

Bennett, J.S., Griffiths, D.T., McCarthy, N. D., Sleeman, K. L., Jolley, K. A., Crook, D. W. and Maiden, M. C. J. (2005) Genetic diversity and carriage dynamics of Neisseria lactamica in infants. *Infection and Immunity* **73**: 2424-2432. https://doi.org/10.1128/iai.73.4.2424-2432.2005