Clinical Trial Protocol

STRONGER SAFE: PHASE II

A within-subject laboratory and field trial to test the use of commercially available insect repellents against contact from *Musca sorbens*, the putative vector of trachoma

Version 2.3, 12th December 2018
Wellcome Trust

London School of Hygiene & Tropical Medicine is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance and Integrity Office:

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2 Administrative information

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12th December 2018	2.3	• Ensured OSA and AR were appropriately referred to as

	"co-PI" throughout the document.

4 List of Abbreviations

Ct	*Chlamydia trachomatis*
FHF	The Fred Hollows Foundation
FMOST	Federal Ministry of Science and Technology
GTMP	Global Trachoma Mapping Project
LSHTM	London School of Hygiene & Tropical Medicine
NTD	Neglected Tropical Diseases
TF	Follicular Trachoma
WHO	World Health Organization
DEET	*N*,*N*-diethyl-3-methylbenzamide
PMD	para-Menthane-3,8-diol
IR3535	Insect Repellent 3535
CPT	Complete Protection Time
mCPT	Median Complete Protection Time
MED	Median Effective Dose
MET	Median Effective Time
MSDS	Material Safety Data Sheet
BSA	Body Surface Area
PSF	Participant Screening Form
GDPR	General Data Protection Regulation
ODK	Open Data Kit
PI	Principle Investigator
CI	Chief Investigator
SC	Steering Committee
DMC	Data Monitoring Committee
GLP	Good Laboratory Practice
GCP	Good Clinical Practice
ORHB	Oromia Regional Health Bureaux
FMHACA	Food, Medicine and Health Care Administration and Control Authority (Ethiopia)
RGIO	Research Governance and Integrity Office (LSHTM)
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
OPP	Office of Pesticide Programs

5 Summary

Background: Trachoma is the commonest infectious cause of blindness worldwide, which leads to considerable ocular morbidity in children and adults. It is caused by ocular infection with the bacterium *Chlamydia trachomatis* (*Ct*). Trachoma is endemic in many areas of Ethiopia, which has the highest burden of this disease globally. Trachoma control requires implementation of the WHO-endorsed SAFE strategy: **S**urgery for trichiasis; **A**ntibiotics to treat infection; **F**acial cleanliness and **E**nvironmental hygiene to reduce transmission. Although SAFE has been successful in reducing disease burden in many areas of the world, there is growing evidence that for hyperendemic regions, particularly in Ethiopia, implementation of SAFE (even under research study conditions) does not have the anticipated effect in reducing and eliminating disease.

Clinical Trial rationale: *Musca sorbens*, a fly that feeds from ocular and nasal discharge on humans, is thought to be the vector of trachoma. As part of Stronger-SAFE Phase II we are developing methods of fly control that specifically target this species, in the hope of interrupting *Ct* transmission. To our knowledge, the use of commercially available insect repellents has never been tested for prevention of *Musca sorbens* fly-eye contact (i.e. nuisance and landing in the peri-ocular area). Given the likely necessity for prolonged and/or high frequency fly-eye contact for *Ct* transmission, the reduction of these contacts through the use of fly repellents presents an exciting opportunity for disease control.

Clinical trial objective: To measure the protective efficacy (personal protection) of repellent products, by comparison of the inhibition of *Musca sorbens* contacts on participants before and after their application.

Study type: This is a within-subject, non-masked, trial of the use of commercially available insect repellents against *Musca sorbens*, with two consecutive participant groups in the laboratory and in the field, and a primary endpoint of measuring the protective efficacy of each repellent product.

Study design:

1. Laboratory trials
    a. Target sample size: 17 participants (all participants test all product iterations)
    b. Stage 1. Protective Efficacy. Determining the protection of repellent products. Only those products/concentrations that protecting against at least 30 % of fly contact will be carried on to stage 2.
    c. Stage 2. Persistence. The persistence of effect will be measured over a six-hour period. For slow-release wearable repellent technologies, this period will be extended for follow-up at 1, 2, 3 and 4 weeks. Estimations of persistence will allow final selection of repellent products/concentrations to be tested in the field trials
2. Field trials
    a. Target sample size: 29 participants per study arm (each participant tests only one product iteration), 10 participants in the Pilot Phase
    b. Up to four groups (study arms) will test the effectiveness of only one topical repellent or slow-release wearable repellent device, at one concentration, against a control group who will receive no intervention.

Intervention: Repellent products will be chosen from: DEET (*N*,*N*-diethyl-3-methylbenzamide), IR3535 (3-[N-butyl-N-acetyl]-aminopropionic acid ethyl ester), Picaridin (2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester); PMD (para-Menthane-3,8-diol) or permethrin (m-Phenoxybenzyl)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate). Products tested will be either (1) topical repellents, or (2) in long-lasting, plastic formulations of repellents that can be worn on the body (wearable repellent technologies). The insect repellent synergist Vanillin (4-Hydroxy-3-methoxybenzaldehyde) may be added to the long-lasting plastic formulations, to improve the duration of protection.

Main study outcomes/endpoints: Protective Efficacy, Complete Protection Time, Median Effective Time and Median Effective Dose

Key inclusion criteria (Laboratory trials, LSHTM):
Ages eligible for study: 18 years and ≤ 65 years
Sexes eligible for study: both
Health of volunteers: full health only, no known adverse reactions, or evidence at screening of adverse reactions, to the commercially available repellents DEET, PMD, IR3535, Picaridin or Permethrin, or to Vanilla
Inclusion criteria: willing to allow 100 laboratory-reared *Musca sorbens* flies to land and crawl on their arm, during the modified arm-in-cage assay, for periods of up to ten minutes at a time, as much as possible without disturbing fly behaviour.

Key inclusion criteria (Field trials, Ethiopia):
Ages eligible for study: 3 years and ≤ 12 years
Sexes eligible for study: both
Health of volunteers: full health only, no known adverse reactions, or evidence at screening of adverse reactions, to the commercially available repellents DEET, PMD, IR3535, Picaridin or Permethrin, or to Vanilla
Inclusion criteria: willing to sit still on a chair outside their house, for sequential periods of up to ten minutes, allowing wild fly contact and landing on the body and face, as much as possible without disturbing fly behaviour.

Nature and extent of the burden and risks associated with participation:

Benefits: Participants in the laboratory trial will receive no benefits from participation in the trial. Participants in the field trial will have the opportunity to have their vision and eyes checked by the Stronger-SAFE project team, and will receive appropriate referral for identified problems. There are no further benefits expected for any participants.

Burden: In the laboratory trials, participants will be required to make repeat visits to the LSHTM testing facility, to test each product and product formulation. During visits, they will be required to sit still for ten-minute observation periods, allowing flies to crawl freely over their forearm and hand. In the field trials, the participant's face will be observed and filmed for ten-minute periods. During this time, the participant will be required to sit motionless and allow flies to crawl freely over their face and around their eyes, nose and mouth. In this study setting this burden would be considered 'the norm', with individuals rarely bothering to brush away flies due to their extreme persistence and prevalence.

Risks: There is a small risk of skin irritation or reaction following application of the repellent product. In the field trials, topical repellents must be applied to the face due to the nature of the target species (eye-seeking flies). The topical repellent active ingredients that will be tested topically have been chosen because they do not carry a specific risk of eye irritation beyond what would reasonably be expected through application of a chemical product. Topical repellents will be applied at a set distance away from the eyes, nose and mouth. Wearable repellent technologies will be formulated to contain insect repellent at doses within published limit of safe application. Because the repellent product will be formulated into plastic, most likely low density polyethylene (LDPE) or high density polyethylene (HDPE), the amount of product that is released onto the skin will be considerably lower than that which is experienced via topical application of a cream. Safety information regarding the repellent active ingredients used in the trial have been assessed, material safety data sheets (MSDS) and labels have been read to be sure they are safe for human use. Participants will be exposed to contacts by *Musca sorbens*. In laboratory trials, *Musca sorbens* will have been reared in captivity for over six generations and carry no risk of Ct transmission. Participants will only be exposed to fly contact on their arms, and after completion of testing, will immediately be instructed to wash their arm. Therefore, the modified arm-in-cage assay presents only negligible risk. In field trials, testing will occur outside the participant's houses, therefore participants will not be exposed to any greater risk from fly contact than that which they experience day-to-day.

6 Contributorship

AR, JL, MB, AL, AB and AC conceived of the study. AR and JL initiated the study design and MB, AL, OS, AC and AB assisted with implementation. DM provided statistical expertise in clinical trial design and AR is conducting the primary statistical analysis. All authors contributed to refinement of the study protocol and approved the final manuscript.

7 Sponsor and Funder

The Wellcome Trust (funder) had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results. The sponsor (LSHTM), principal investigators and collaborators accept full responsibility for all aspects of the study.

SPONSOR

London School of Hygiene & Tropical Medicine will act as the main sponsor for this study. Delegated responsibilities will be assigned locally.

INDEMNITY

London School of Hygiene & Tropical Medicine holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this trial

AUDITS AND INSPECTIONS

The study may be subject audit by the London School of Hygiene & Tropical Medicine under their remit as sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

8 Organisational structure and responsibility

Chief investigator: Prof. Matthew Burton
Co-Principal investigator (lab) and Laboratory Project Manager: Dr Ailie Robinson
Co-Principal Investigator (field) and Field Project Manager: Mr Oumer Shafi Abdulrahman
Preparation of protocols and revisions: Dr Ailie Robinson, Prof. James Logan, Prof. Matthew Burton, Dr Anna Last, Dr Adam Biran, Ms. Alex Czerniewska
Organising steering committee meetings: Dr Ailie Robinson
Publication of study reports: Dr Ailie Robinson, Prof. James Logan, Prof. Matthew Burton, Dr Anna Last
Steering Committee (SC): Dr Ailie Robinson, Prof. James Logan, Prof. Matthew Burton, Dr Anna Last, Mr Oumer Shafi Abdulrahman, Dr Adam Biran, Dr Katie Greenland, Dr Esmael Ali, Dr Aalbertus Versteeg, Ms. Alex Czerniewska. Responsible for study planning, for reviewing the progress of the study, agreeing any changes to the protocol if and when required, and ensuring the smooth running of the study. Will report any SAEs [Serious adverse events] to the LSHTM ethics committee.
Data Monitoring Committee: Dr Vanessa Chen-Hussey, *to be confirmed*.
Agreement of final protocol: SC
Recruitment of participants and liaising with laboratory co-PI: Dr Ailie Robinson
Data manger: Dr Ailie Robinson
Lead investigator: Dr Ailie Robinson

9 Introduction

9.1 Trachoma

Trachoma, a Neglected Tropical Disease (NTD), is the commonest infectious cause of blindness globally, affecting some of the world's poorest communities(1). Trachoma is caused by repeated ocular infection with the bacterium *Chlamydia trachomatis* (*Ct*). Active trachoma begins in childhood with recurrent episodes of follicular conjunctivitis (TF). Chronic inflammation results in immunologically mediated conjunctival scarring and in-turned eyelashes scratching the eye: trichiasis. Eventually sight is lost from irreversible corneal opacification.

Trachoma is currently endemic in 42 countries. The latest estimates from the Global Trachoma Mapping Programme (GTMP) suggest that 180 million people live in trachoma endemic areas and 3.2 million people have trachomatous trichiasis (2). Around 2.2 million people are visually impaired, of whom 1.2 million are blind (3). More than 80% of the burden of active trachoma is concentrated in 14 countries, mainly in the Sahel of West Africa and savannahs of East and Central Africa, where water supplies are often scarce(2).

9.2 Trachoma treatment, prevention and control

Trachoma control requires community-wide measures. The World Health Organization (WHO) Alliance for the Global Elimination of Trachoma by 2020 (GET2020) recommends the SAFE Strategy: **S**urgery for trichiasis, **A**ntibiotic to treat *Ct* infection, **F**acial cleanliness and **E**nvironmental improvements to suppress transmission(1). Many endemic countries are implementing SAFE, and there has been a major effort to scale up activities, aiming to eliminate trachoma by 2020(2).

Currently, the antibiotic component involves mass drug administration (MDA) with oral azithromycin to all community members older than six months. This is given as a single, annual dose, initially for 1-5 years, before reassessing the district-level TF prevalence in 1-9 year olds and deciding whether MDA can be discontinued(4). The F&E components are much more variable in content and application. If F&E are implemented at all, it usually involves improving water access, sanitation and hygiene (WASH) and fly-control(1).

Unfortunately, there is now growing evidence, particularly from hyperendemic regions (>20% TF), that current approaches are not having the anticipated impact on infection and disease(5–8). This is a significant threat to the timely elimination of trachoma. Over 44 million live in districts with >30% TF (GTMP data). In hyperendemic areas, current antibiotic schedules appear insufficient to reliably achieve long-term control after treatment completion. For example, in Ethiopia, which has the greatest trachoma burden, despite seven years of annual or biannual high-coverage MDA, the prevalence of TF remains well above threshold for continuing MDA(5). Data on *Ct* after repeated MDA rounds in hyperendemic settings indicates that reliable long-term control is not consistently achieved, with re-emergence of infection being typical(6, 8).

It is unknown which, if any, F&E measures, as applied programmatically, suppress *Ct* transmission. The trachoma literature is replete with studies (including several conducted by the applicants) which report associations between active trachoma and/or *Ct* infection and WASH indicators

(water and latrine access), fly-eye contact and clean faces. Based on these associations a recent meta-analysis concluded there is "strong evidence to support F&E components of SAFE"(9). However, we disagree with this conclusion. What has been demonstrated are associations, rather than causal relationships. There are few randomized-controlled trials in this area, which have demonstrated limited or no effect(10–15). Recent Cochrane Reviews of F&E intervention trials concluded there is currently little or no evidence that the tested interventions significantly impacted on trachoma(16, 17).

Moreover, our understanding of how *Ct* is transmitted within endemic communities is largely based on supposition. We believe that endemic trachoma is sustained by ongoing person-to-person *Ct* transmission, probably through a combination of direct contact and indirect transmission on fomites and flies (*Musca sorbens*). However, detailed studies investigating potential transmission routes and their relative importance have never been conducted. Therefore, we do not currently have a clear, evidence-based understanding of transmission biology or its socio-behavioural determinants, on which to base rational decisions about public health F&E interventions to eliminate trachoma.

There are at least three critical issues:

1. Routes of *Ct* transmission and their relative importance are poorly defined, as detailed studies have never been conducted, making it hard to focus F&E interventions.
2. The F&E intervention evidence base is very limited: there are few published randomized-controlled trials, which have demonstrated limited or no effect, to guide programmes.
3. Particularly in hyperendemic areas, current azithromycin schedules, with or without F&E, appear insufficient to control infection and disease.

To address these issues, we propose a sequence of interrelated studies in Ethiopia, conducted through a multi-disciplinary collaboration in three Phases, which will develop and test enhanced A, F & E strategies for trachoma elimination: **Stronger-SAFE**. In this protocol, we outline aspects of **Phase II** of this programme.

9.3 Trachoma in Ethiopia

Ethiopia remains the country with the greatest trachoma burden(2). It is estimated that 30% of Africa's trachoma burden is in Ethiopia. More than 80% of its population of 90 million live in rural areas and 37% live on less than a dollar a day(18). Half the population travel significant distances to access safe drinking water, with 12 percent of the population still relying on untreated surface water(19). A national survey conducted in Ethiopia in 2010 showed that access to water supply and sanitation was 52% and 63% respectively(20). These environmental and living conditions are believed to create the ideal situation for trachoma to flourish.

Recently collected Global Trachoma Mapping Project (GTMP) data from Ethiopia show that more than 76 million people are at risk of trachoma and the prevalence of TF in 1-9 year olds (TF1-9) ranges from 0.2% to 73.4% (Figure 1). In Oromia, both active trachoma and trichiasis are significant public health problems. The most recent GTMP data published for this region shows an estimated overall prevalence of TF1-9 of 23.4% across 252 districts(21). In 46% of surveyed districts, TF1-9 prevalence was >30% (Figure 2) in 126 of 252 districts(22). Disabling sight loss and pain from trichiasis predominantly affects women. It has been estimated that trachoma causes up to US$ 8 billion/year productivity loss, a burden that falls on some of the poorest

communities(23). Our recent work from Ethiopia found households of individuals with trichiasis are significantly poorer than their unaffected neighbours(24). Moreover, trichiasis has a profound impact on quality of life(25).

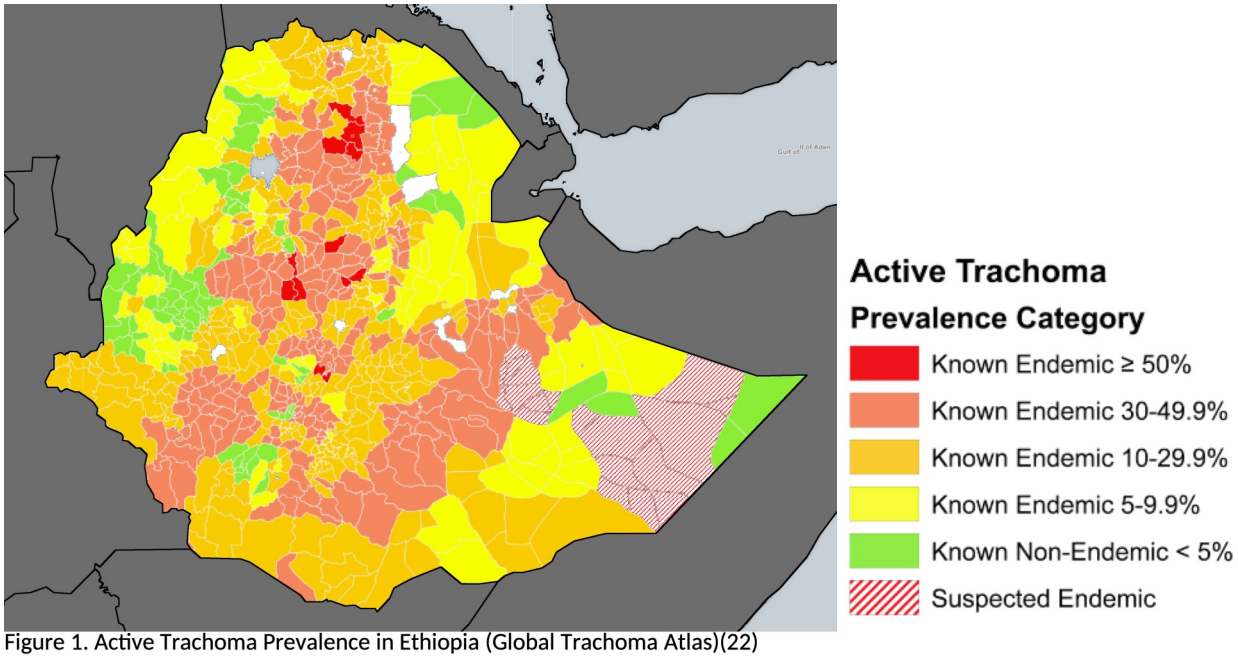

Figure 1. Active Trachoma Prevalence in Ethiopia (Global Trachoma Atlas)(22)

Ethiopia is working towards eliminating trachoma by 2020 and began implementing the SAFE strategy as part of national policy in 2003. This has focused on the provision of improved trichiasis surgery, MDA and the distribution of public health messages by radio, video, and printed material. From 2001-2015 more than one million trichiasis surgeries were performed, over 170 million doses of azithromycin were given through MDA and more than 24 million latrines were built. Despite these encouraging efforts, trachoma remains a public health problem in many regions of the country, and the burden of disease is far above the elimination targets set by WHO. In many of these communities, despite seven years of annual or biannual high-coverage MDA, the prevalence of TF remains well above threshold for continuing MDA. Data on *Ct* prevalence after repeated rounds of MDA in hyperendemic settings such as Ethiopia, indicate that reliable long-term control is not consistently achieved, with gradual re-emergence of infection being typical(6).

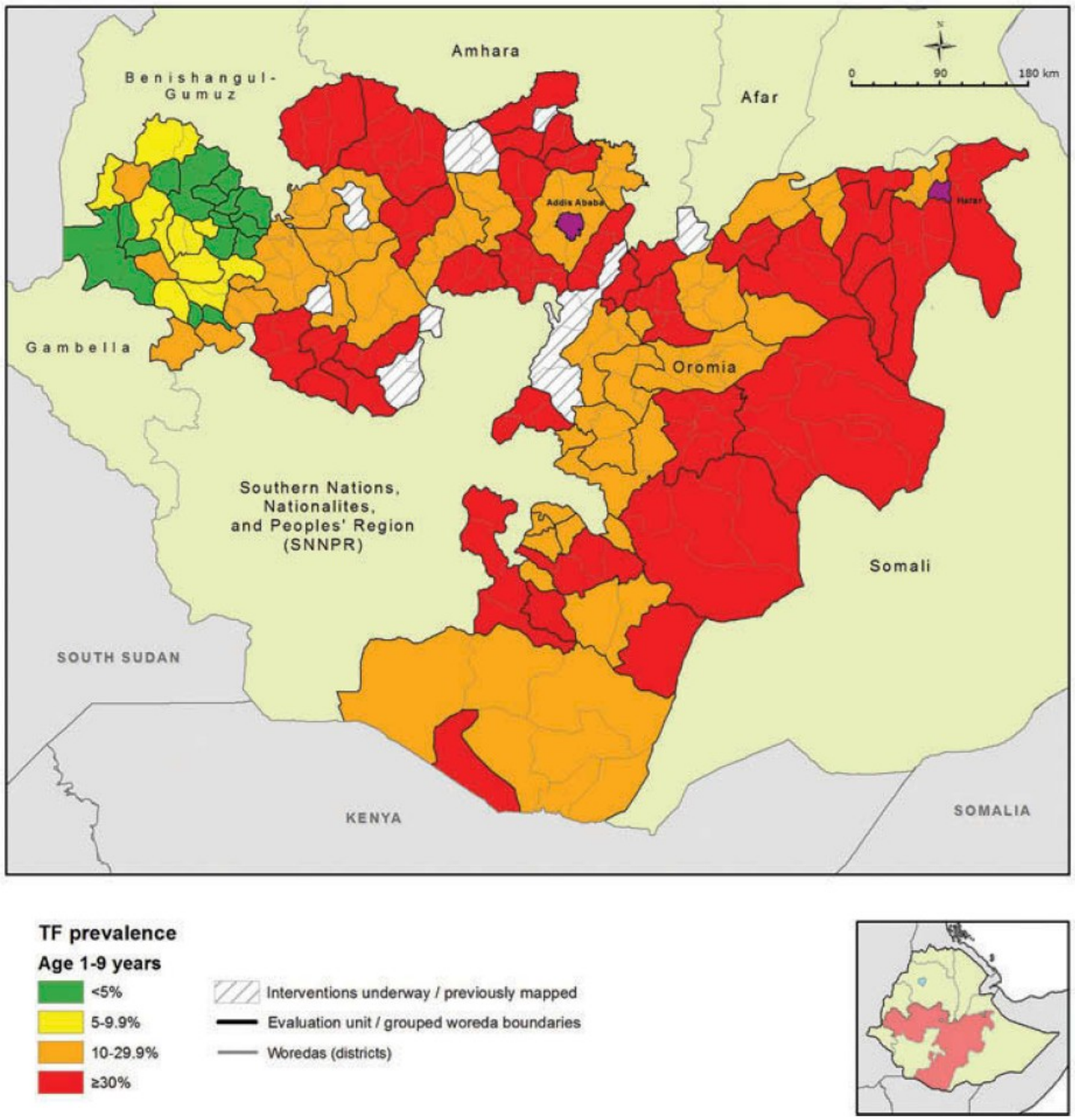

Figure 2. Prevalence of TF in 1-9 year olds by evaluation unit from 2012-2014 (GTMP)(21)

9.4 Flies and Trachoma

Flies are likely to contribute to *Ct* transmission. *Ct* can be cultured from guts and limbs of *Musca domestica* fed on *Ct*-infected egg yolk(26). Using a tightly controlled guinea pig trachoma model, *Chlamydia psittaci* was transmitted by flies from infected to uninfected eyes(26). Infection was established consistently if the time between flies feeding on infected guinea pig ocular secretions and being exposed to uninfected guinea pigs was under one hour. Although there is some evidence that flies are involved in transmission, it is poorly understood.

In The Gambia, *Musca sorbens*, which breeds most successfully in human faeces, accounts for >90% of fly-eye contacts(27, 28). In the "Flies and Eyes" study, intensive control through long-term insecticide spraying was associated with a significant reduction in active trachoma (infection not

tested)(12). In the same study, installing pit latrines was associated with a significant reduction in fly-eye contacts and a non-significant reduction in active trachoma(12). However, when azithromycin MDA was combined with intensive insecticide spraying in Tanzania, there was no additional benefit(11). Two studies tested *M. sorbens* caught leaving faces of Ethiopian children for *Ct* by PCR; 15-23% of flies were positive(29, 30). These data suggest *M. sorbens* may be a trachoma vector, however, its relative importance probably varies by setting. Interventions to control *M. sorbens* tried to date have met with variable success; intensive insecticide spraying is probably not a sustainable control measure.

Our recent Gambian studies found *M. sorbens* is strongly attracted to odours produced by human faeces. Compared to other sources of excrement (dog, cow, horse), human excrement was up to sixty times more attractive. Volatile odours were collected from faeces and analysed by gas chromatography, mass-spectrometry and electrophysiology to identify chemicals detected by *M. sorbens*. Several putative attractants were identified in the odour of human faeces. The next step is to test these odours in behavioural experiments, with the aim of developing traps, which will locally suppress *M. sorbens* populations around human habitations. We have developed methods to establish *M. sorbens* laboratory colonies for behavioural studies. We have pilot-tested video observation of fly-eye contact for behaviour and transmission studies.

Attractant and repellent technologies could be combined to create a “push-pull” strategy to reduce vector-host contact and attract flies to lethal oviposition traps to supress populations. More research is warranted in this area, particularly as our formative research indicates that control of flies would be a particularly popular public health measure. The Logan entomology research group at LSHTM group works in partnership with industrial collaborators, to investigate long-lasting wearable arthropod repellent technologies including clothing, wrist bands, necklaces and footwear.

To date there has been little investigation of the potential contribution of flies in the transmission of trachoma in Ethiopia, this warrants further study as this may be an important component.

9.5 Rationale for the use of repellents against *Musca sorbens*

The use of insect repellents is widespread world-wide to prevent nuisance biting by non-vector species, and to prevent disease transmission by vectors in disease-endemic regions. Although the use of plants with repellent qualities, either by burning leaves or presenting fresh foliage (31, 32), is prevalent in many regions, commercially available topical repellents are rarely used by people in low-income and disease-endemic countries. This is because of cost, availability, and the practicalities of a product that requires repeat application. As such, there are limited studies on the effectiveness of repellent personal protection against prevention of disease, however, repellents have been used successfully to control arthropods of public health significance but which do not control disease, including lice (33) and the chigoe flea (34, 35). However, there is growing interest in the use of repellents as personal protection from disease transmission, particularly around the use of insecticide-treated clothing (36–39), which can repel biting insects. Insecticide treated clothing has been shown to provide protection from both malaria and leishmaniasis (40). Another study looked at the use of insecticide-treated headscarves for Afghan women in a Pakistani refugee camp, and found a reduction in the incidence of malaria in people under 20 years old (41).

As well as transmitting *Chlamydia trachomatis*, *Musca sorbens* flies can cause severe distress due to their eye-seeking behaviour (Figure 3). Therefore, reducing the number of *M. sorbens* face contacts would not only contribute towards breaking the transmission cycle of Ct, but would also alleviate distress in regions where *M. sorbens* are found. For these reasons, it is possible that personal protection against *M. sorbens* by insect repellents could be highly successful, as the immediate benefit of reduced face contact would encourage continued uptake of this intervention.

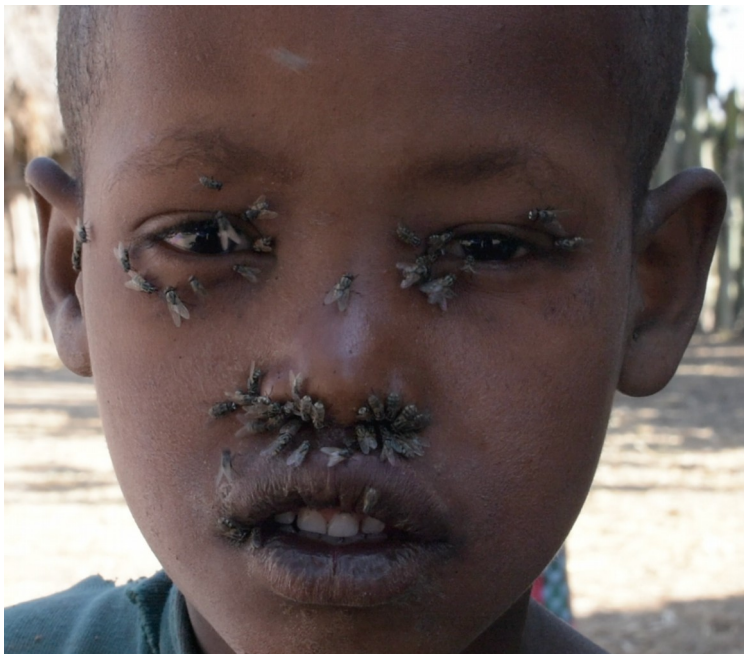

Figure 3. Eye-seeking behaviour of *Musca sorbens*. Photo taken by A. Robinson, Faji Gole, Ethiopia, January 2018, reproduced with permission.

Relative to other vectors of disease, very little is known about the biology and ecology of *M. sorbens*, although limited studies are available (27, 28, 42–45). Particularly, the only *M. sorbens* control measures that have been robustly studied are that of insecticide, and breeding site/larval source management (46). However, other closely related species are better understood, and repellents have been used with mixed success against the bush fly *Musca vetustissima* (47, 48), the face fly *Musca autumnalis*, and the housefly *Musca domestica* (48–50). Insecticide-impregnated ear-tags have also been developed for use in cattle, against horn- and face-flies (51).

10 Research hypothesis

Commercially available insect repellent products can be used to decrease contact to the face, particularly the eyes, nose and mouth, by the eye-seeking fly *Musca sorbens*. The protection afforded by insect repellents will prevent transmission of *Chlamydia trachomatis* by infected flies, as well as reducing the nuisance caused by this species.

11 Choice of comparators

It is well-established that individuals vary in their attractiveness to biting insects. A number of factors are thought to contribute to this variation in attractiveness, including body weight and/or

surface area (52), hormones (53), genetic factors (54) or disease (55, 56). Although *Musca sorbens* flies do not imbibe a blood meal, they are attracted to the face, and this attraction is presumably mediated via cues including odour and vision, that are highly person-specific. Further, there is evidence to suggest that flies are more attracted to individuals with ocular or nasal discharge, which is in turn influenced by the presence of trachoma. It is therefore reasonable to speculate that for a multitude of reasons some individuals are more attractive to *M. sorbens* flies than others, and therefore a within-subject trial, which controls for such variation, is the optimal study design.

12 Study Objectives

12.1 Primary Objective

To measure the protective efficacy (personal protection) of repellent products, by comparison of the inhibition of *Musca sorbens* contacts on participants before and after their application.

The products will be some or all of the following insect repellents: DEET (N,N-diethyl-3-methylbenzamide), IR3535 (3-[N-butyl-N-acetyl]-aminopropionic acid ethyl ester), Picaridin (2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester); PMD (para-Menthane-3,8-diol) or permethrin (m-Phenoxybenzyl)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate). Repellents will be applied 1) topically on the skin, or 2) in long-lasting, plastic formulations of that can be worn on the body (wearable repellent technologies). The insect repellent synergist Vanillin (4-Hydroxy-3-methoxybenzaldehyde) may be added to the long-lasting plastic formulations, to improve the duration of protection.

12.2 Secondary Objectives

1. To compare the duration of protection offered by different repellent products using the median Complete Protection Time (mCPT)
2. To compare the effectiveness of protection offered by different repellent products using the median effective dose and median effective time
3. To assess the acceptability of the repellent products tested in the field trials using qualitative data from participants.

13 Trial Design

This is a within-subject, non-masked, trial of the use of commercially available insect repellents against *Musca sorbens*, with two consecutive participant groups in the laboratory and in the field, and a primary endpoint of measuring the protective efficacy of each repellent product.

The trial is within-subject to allow comparison of *M. sorbens* contacts on the same participants both before (control) and after (test) application of the repellent or repellent device. This is to mitigate any possible inter-individual attractiveness effects. Control sampling will be conducted before test sampling, to preclude contamination of the control sampling by the test sampling. For this reason, the trial is not masked.

13.1 Laboratory trial

In preliminary laboratory clinical trials in London, 17 participants will test all products that have been found to exhibit repellency to *Musca sorbens* in benchmarking laboratory studies. These will be chosen from: DEET (*N*,*N*-diethyl-3-methylbenzamide), IR3535 (3-[N-butyl-N-acetyl]-aminopropionic acid ethyl ester), Picaridin (2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester); PMD (para-Menthane-3,8-diol) or permethrin (m-Phenoxybenzyl)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate). Products tested will be either (1) topical repellents, or (2) in long-lasting, plastic formulations of repellents that can be worn on the body (wearable repellent technologies). The insect repellent synergist Vanillin (4-Hydroxy-3-methoxybenzaldehyde) may be added to the long-lasting plastic formulations, to improve the duration of protection.

As all participants will test all products and all concentrations, this study is non-randomised. The combination of product and product format to be tested will be determined before the study commences. Participants will be asked to place their arm in a cage with 100 *Musca sorbens* flies, and the behaviour of the flies on the surface of the arm and hand will be filmed for ten minutes. This modified arm-in-cage assay has been developed specifically for use with *Musca sorbens*.

There will be a two-stage selection process to determine which repellent products, at which concentration (dose), should be carried forward to the field trial. In the first stage, a single measure of Protective Efficacy (PE) will be used to determine protection, by measuring the duration of fly-arm contact after application of a repellent product (test measurement) relative to that before application of the product (control measurement). By only selecting repellent products/concentrations that protect against at least 30 % of fly contact immediately following application (at time zero), those with little or no effect will be disregarded.

In the second stage and using the same participants, those repellent products/concentrations that demonstrated at least 30 % PE will be measured for the persistence of effect, over a six-hour period. For the wearable repellent devices, tests will further be repeated at one, two, three and four weeks later. The duration of fly contact in a modified arm-in-cage assay will again be used. This stage will allow estimations of persistence including the Median Effective Dose (ED_{50}), the Median Effective Time (ET_{50}) and the Complete Protection Time. Estimations of persistence will allow final selection of repellent products/concentrations to be tested in the field trials.

13.2 Field trial

In the field clinical trial, eligible participants will be randomised between groups (study arms), each of which will test only one topical repellent or slow-release wearable repellent device, at one concentration. A maximum of four groups will be tested in addition to a control group, and each group will contain 29 children between the ages of three and 12 years. The additional control group, receiving no product, will allow for temporal comparison of fly contact across a day of testing.

The combination of product and product format to be tested will be determined before the study commences. The PE will be determined by measuring the frequency of fly-eye, fly-nose, fly-mouth and fly-face contacts by *Musca sorbens* after application of the product, in a 'field' environment where these flies are naturally present at high density. Control measurements will be taken of fly contact on each participant prior to this. Test measures will be repeated over a period of six hours to determine the ET_{50}, and for wearable repellent devices, participants will continue to wear the

product and tests will be repeated at one, two, three and four weeks later. Topical repellents will be applied by the Stronger-SAFE field team nurses, who will also demonstrate how to wear the repellent devices. A qualitative assessment of acceptability and barriers to use will be carried out at the end of the trial.

14 Study setting and populations

14.1 Age of participants

This clinical trial has two consecutive participant groups, firstly adults in the laboratory trials and then children of age three to 12 years in the field trials. The first study will test proof-of-concept for the use of insect repellents against *Musca sorbens*, will narrow down repellent candidates, and will not replicate a naturalistic setting. The second study will take place in a trachoma-endemic setting, where *Musca sorbens* are prevalent and likely contributing to disease transmission. Because fly harassment around the eyes, nose and mouth is not a problem that adults experience, it would neither be possible nor naturalistic to conduct the field study using adults.

14.2 Laboratory trial (London School of Hygiene & Tropical Medicine)

Laboratory studies will be conducted in specialised, Good Laboratory Practice (GLP)-accredited insect testing facilities at LSHTM. A colony of field-collected, but laboratory-reared, *Musca sorbens* are maintained by the lead investigator in insectary facilities at LSHTM, these will be used for all laboratory trials. We will enrol adults (>18 years) of both sexes, among staff and students of LSHTM, to this component of the trial.

14.3 Field trial (Oromia, Ethiopia)

Field studies will be carried out in one woreda (district) in the West Arsi Zone of Oromia, Ethiopia, in the same approximate locality that the other Stronger-SAFE Phase II studies are being conducted, but in villages (kebeles) that have not previously been enrolled to any other Stronger-SAFE study component. Kebeles will be chosen where TF prevalence is believed to be low (TF1-9<40%). We will perform trachoma screening in the selected kebeles to confirm TF prevalence. We will select low prevalence areas as these studies do not incorporate clinical or *Ct* prevalence outcomes, requiring only fly populations, therefore we will aim to set the study site where there is minimal disease transmission but a high abundance of *Musca sorbens*. We will conduct a preliminary assessment of the suitability of study sites by visiting and observing the extent of local fly-eye nuisance among children. We will enrol households with children aged three to 12 years, as they are at increased risk of TF relative to adults, and also tend to experience higher levels of face fly nuisance. This field study location will provide an excellent context for informing on the wider applicability of the study results, due to both the very high TF prevalence rates (21) and fly population densities (29) experienced in this area. Small-scale field repellency trials will be conducted at the participant's houses.

15 Trial eligibility and withdrawal criteria

Participants will be healthy individuals, and will be included in the study if they meet all of the following criteria:

15.1 Laboratory trial eligibility criteria

1. Participant is aged 18 years and ≤ 65 years and in good health
2. Participant has a good understanding of the procedures of the study and agrees to abide to these procedures
3. Participant is able to communicate well with the investigator, and attend the laboratory for all aspects of the laboratory studies
4. Participant has no known adverse reactions, or evidence at screening of adverse reactions, to the commercially available repellents DEET, PMD, IR3535, Picaridin or Permethrin, or to Vanilla
5. Participant has no known history of skin allergies or hypersensitivity to topical creams
6. Participant agrees to a pre-trial skin reactivity test for all the repellents that will be used in the trial
7. If in the event of the participant experiencing an adverse reaction to a repellent during the trial, the participant agrees to inform his/her general practitioner and seek appropriate treatment if necessary
8. Participant is willing to allow laboratory-reared *Musca sorbens* flies to land and crawl on their arm, during the modified arm-in-cage assay, for periods of up to ten minutes at a time
9. Participant agrees not to use any perfumed or scented product, including bathing products, for a 24-hour period before each laboratory session
10. Participant has signed informed consent
11. Participant is not a smoker, and will agree to refraining from smoking for the 12 hours before each laboratory trial

15.2 Field trial eligibility criteria

1. Participant lives in the designated study site
2. Participating households must be within a one-hour drive of Feya General Hospital
3. Participant considers themselves to be in good health, as does the parent or guardian
4. Participant is aged 3 years and ≤ 12 years
5. Participant has a good understanding of the procedures of the study and agrees to abide to these procedures
6. The parent or guardian of the participant has a good understanding of the procedures of the study and agrees to abide to these procedures
7. Participant is able to communicate well with the investigator or fieldworker who is conducting the study
8. Participant has no known adverse reactions to the commercially available repellents DEET, PMD, IR3535, Picaridin or Permethrin, or to Vanilla
9. Participant has no known history of skin allergies or hypersensitivity to topical creams
10. Participant agrees to a pre-trial skin reactivity test for all the repellents that will be used in the trial
11. If in the event of the participant experiencing an adverse reaction to a repellent during the trial, the participant can request medical advice from the Stronger-SAFE field team nurses if they wish
12. Participant is willing to sit still on a chair outside their house, for sequential periods of up to ten minutes, allowing wild fly contact and landing on the body and face, as much as possible without disturbing fly behaviour
13. Participant agrees not to use any perfumed or scented product, including bathing products, for a 24-hour period before each laboratory session

14. Able and willing to give fully informed assent
15. The parent or guardian has signed informed consent
16. The participant does not become unacceptably upset during the procedures

15.3 Participant withdrawal

Participants can stop at any time without giving a reason for withdrawing. Data collected to the point of withdrawal will be used in the analysis of the study, unless the participant requests that their data is not used, in which case it will be removed from the database. Participants may also be removed at the discretion of the Chief Investigator, where continued participation may affect the safety of the participant or where there is a development of any condition which might interfere with study participation.

15.4 Participant retention

Once participants are enrolled to either the laboratory or field clinical trial, both study sites will make every reasonable attempt to ensure that these participants are followed for the entire study period, when repeat observations are necessary over a duration of one month for the wearable repellent devices.

For the laboratory clinical trial, the loss-to-follow-up over this month is expected to be low, and 5 % loss-to-follow-up has been allowed for the laboratory trial sample size (+ one child). For the field clinical trial, loss-to-follow-up over that month is expected to be higher, as it may be harder to locate young children and ensure that they are at home on the required days. Therefore, a 25 % loss-to-follow-up has been allowed for the field trial sample size (+ six children). Fieldworkers at this study site will be responsible for developing and implementing local standard operating procedures to achieve this level of follow-up.

16 Interventions

16.1 Investigational products

16.1.1 Topical insect repellent products

One or more of three insect repellent products (Table 1), previously determined by laboratory experiments at LSHTM to exhibit potential repellency to *Musca sorbens*, will be tested.

Table 1. Insect repellent products that will be applied topically will be selected from these three actives

Generic name	Repellent active ingredient	CAS number	Manufacturer
DEET	*N*,*N*-Diethyl-meta-toluamide	134-62-3	Merck[(1)]
IR3535	3-[N-butyl-N-acetyl]-aminopropionic acid ethyl ester	52304-36-6	Merck [(2)]
Picaridin	2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester	119515-38-7	Alfa chemistry[(3)]

(1) https://www.sigmaaldrich.com/catalog/substance/deet1912713462311?lang=en&region=GB

(2) https://origin-webqws.sial.com/catalog/product/sial/34524?lang=en&region=GB&cm_sp=Insite-_-recent_fixed-_-recent5-3

(3) https://www.alfa-chemistry.com/sec-butyl-2-2-hydroxyethyl-piperidine-1-carboxylate-cas-119515-38-7-item-289774.htm

16.1.2 Wearable repellent devices

One or more of five insect repellent products, with the possible addition of vanillin (Table 2), previously determined by laboratory experiments at LSHTM to exhibit potential repellency to *Musca sorbens*, and formulated into long-lasting plastic formulations made of low-density polyethylene (LDPE) or high-density polyethylene (HDPE). In the case of permethrin, the wearable repellent technology will be permethrin-treated fabric, e.g. a permethrin treated neckband. PMD will be allowed in a wearable repellent device, but not as a topical product, because of safety advice against its use on children's faces (57). Vanillin is an organic compound that it is thought may enhance the protection time of insect repellents.

Table 2. Insect repellent products that will be formulated into long-lasting plastic formulations will be selected from these five actives

Generic name	Repellent active ingredient*	CAS number	Manufacturer
DEET	*N*,*N*-Diethyl-meta-toluamide	134-62-3	Merck[(1)]
Oil of Lemon Eucalyptus, PMD	para-Menthane-3,8-diol OR Citriodiol® (64% PMD [a mixture of the cis and trans isomers of p-menthane-3,8-diol) together with a number of minor constituents found in essential oil which enhance the efficacy further])	42822-86-6	Merck [(2)] Citrefine [(3)]
IR3535	3-[N-butyl-N-acetyl]-aminopropionic acid ethyl ester	52304-36-6	Merck [(4)]
Picaridin	2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester	119515-38-7	Alfa chemistry[(5)]
Permethrin	(m-Phenoxybenzyl)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	52645-53-1	Merck[(6)] OR Fagron Hellas[(7)]

*Vanillin (4-Hydroxy-3-methoxybenzaldehyde, CAS number 121-33-5, available from Merck) may be used as a synergist with these repellent actives (https://origin-webqws.sial.com/catalog/product/sial/v1104?lang=en&region=GB)

[(1)] https://www.sigmaaldrich.com/catalog/substance/deet1912713462311?lang=en&region=GB
[(2)] https://origin-webqws.sial.com/catalog/product/aldrich/r751898?lang=en&region=GB
[(3)] https://www.citrefine.com/citriodiol/#what-is-citriodiol
[(4)] https://origin-webqws.sial.com/catalog/product/sial/34524?lang=en&region=GB&cm_sp=Insite-_-recent_fixed-_-recent5-3
[(5)] https://www.alfa-chemistry.com/sec-butyl-2-2-hydroxyethyl-piperidine-1-carboxylate-cas-119515-38-7-item-289774.htm
[(6)] https://origin-webqws.sial.com/catalog/product/sial/45614?lang=en&region=GB
[(7)] https://gr.fagron.com/en-gr

16.2 Application of topical products

All topical products will be applied at the standard laboratory application rate of 1 ml product/600 cm^2.

16.2.1 Laboratory trials application rate

The arm is an estimated 9 % of the adult body surface area (BSA), therefore the forearm and hand can be considered to be 4.5 %. With the average adult BSA of 19,000 cm^2, the surface area of the forearm can be taken to be 855 cm^2. As such, 1.4 ml of solution will be applied to the forearm at the appropriate concentration, never exceeding 20 %.

16.2.2 Field trials application rate

Topical repellent will be applied to a circular area on the cheek. An average diameter of 6 cm would give a surface area of 28.3 cm^2. As such, 0.05 ml (50 μL) of product will be applied to each cheek at the appropriate concentration, never exceeding 20 %. Topical repellents will be applied at least 3 cm (three finger-breadths) below the eye and positioned away from the nose and mouth.

Due to the differences in surface area under observation in the laboratory and field trials, with greater skin surface areas in the former (application on the arm) leading to greater amounts of active ingredient being applied, there is a risk that efficacy in the laboratory will be greater than that experienced in the field. However, it should be emphasised that the aim of the laboratory trials is to inform which repellents can be taken into the field trial for testing, as this is the context in which such a product would be used. Therefore, while both studies are merited, the Protective Efficacy and other outcome measures as calculated in the field trial will be those that are used to inform inclusion of repellents as a fly-control intervention in the Stronger-SAFE Phase 3 RCT.

17 Study outcomes

17.1 Primary outcome measures

The primary endpoint is the protective efficacy of the repellent products. Protective efficacy will be presented as a proportion, by comparing fly contact on the participants following application of the repellent product (test), relative to before application (control) (see section 26.3). Protective efficacy will be determined for all repellent products in both the laboratory and field clinical trials.

17.2 Secondary outcome measures

1. Median Complete Protection Time (mCPT) in laboratory trials only
2. Median Effective Dose (ED_{50}), in laboratory trials only
3. Median Effective Time (ET_{50}), in both laboratory and field trials
4. Acceptability of the repellent interventions among children and their caregivers, in field trials only

18 Participant timeline

A timeline of participant recruitment and enrolment, consent, and completion of the clinical trials is given in Figure 4.

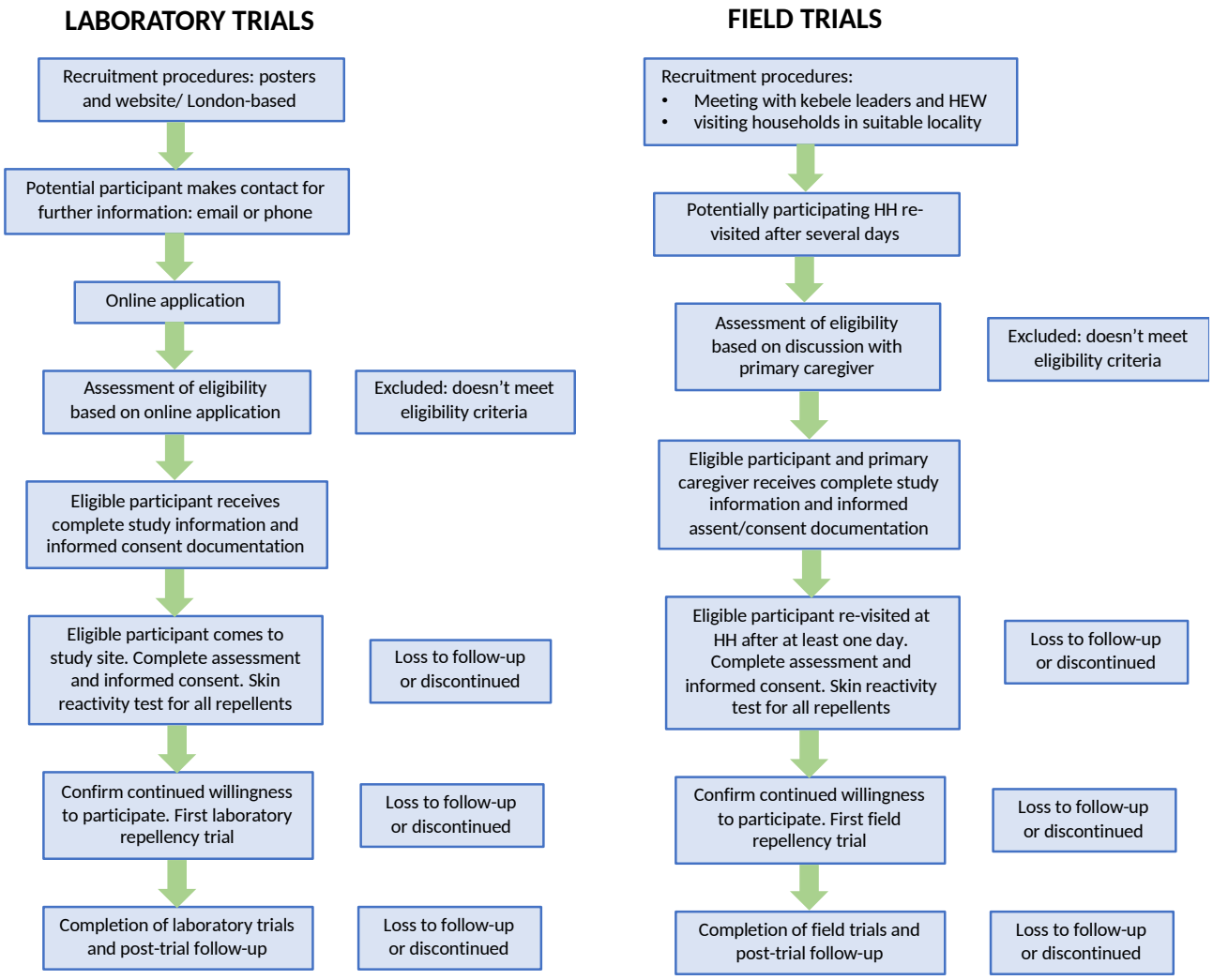

Figure 4. Participant timeline for clinical trials of insect repellents against fly contact by *Musca sorbens*

19 Sample size

19.1 Laboratory trials sample size

For laboratory trials, estimations of the sample size required are dependent on the variability of PE between individuals. As such, a range of sample sizes were calculated that took both this, and variability in the confidence intervals around the estimate, into consideration (Table 3). A conservative estimate of the PE standard deviation of 30 % was chosen, which when allowing for a confidence interval of ±15 % around the estimate, gives a sample of 16 people. When allowing for 5 % loss-to-follow-up, the total sample size will be 17 people. Equal numbers of male and female participants are preferred.

Table 3. Estimation of sample size for determining the Protective Efficacy of repellent products in laboratory trials.

		Standard deviation of Protective Efficacy					
		10 %	15 %	20 %	25 %	30 %	35 %
Margin of error (CI)	5 %	16	35	62	97	139	189
	10 %	4	9	16	25	35	48

	15 %	2	4	7	11	**16**	21
	20 %	1	3	4	7	9	12

For stage two laboratory trials (repellent product persistence), multiple follow-ups on the same individuals will generate more repeat data points. As such, the sample size requirement for PE will be sufficient. If we observe in stage one that variability between individuals was much greater than expected then we will consider increasing the sample size for stage two.

19.2 Field trials sample size

This trial will be powered to detect a protective effect in the intervention (treatment) groups relative to the control groups. To test for 30 % protection (PE) in the intervention arm versus the control arm, assuming a standard deviation of 30% and using 90 % power, 23 children are required in each study group. When allowing for 25 % loss-to-follow-up, the total sample size in each study arm will be 29 people. Equal numbers of male and female participants are preferred.

Not all approximately participants in the wearable repellent device arms of the field trial will be interviewed for intervention acceptability. Based on previous experience, we anticipate that a sample of 15 to 20 child-caregiver pairs, purposively sampled to represent the range of child ages will be sufficient, and further data collection unlikely to yield additional information. However, we will review our data regularly during the data collection process and will adjust the sample accordingly.

20 Enrolment, randomisation and allocation

20.1 Laboratory trials

Participants will be recruited through standard recruitment methods, including emails, posters, leaflets and other advertising routes to staff and students of LSHTM and other members of the public. Participants will be fully informed before the study and it will be made clear that they can withdraw from the study at any time. Participants will be given and asked to read the Participant Information Sheet (Appendix 1) and Product Information Sheet (Appendix 2) which describes the tests which they will take part in, and a consent form (Appendix 3) which must be signed before the test begins. Because in the laboratory trials all participants will test all products and all concentrations, this study is non-randomised.

20.2 Field trials

Prior to approaching members of the communities in which we wish to work there will be initial dialogues with the community leaders and local health officials to introduce the purpose and nature of the research project. Following this, participants will be recruited by visiting households in the study site that are home to children in the correct age bracket. Information about the study will be shared with potential participants by members of the field research team, who have previous experience in the participant information and consenting processes. During the visit, participants will be provided with Information Sheets (Appendices 4 and 5), a Product Information Sheet (Appendix 6) and Informed Consent and Assent forms (Appendix 7). Assent will be sought from the participant, and consent from the primary caregiver. This will be in Afaan Oromo, the

regional language. This will be read to those who are unable read. After verbal explanation of the relevant sections of the Information Leaflet and having the opportunity to ask questions, informed consent will be gained and evidenced by a signature or thumbprint signature (deemed acceptable locally due to high rates of illiteracy), in the presence of the study team and independent witness.

Eligible participants will be randomised equally between the topical repellents and wearable repellent devices found to be protective in laboratory clinical trials, and a control group receiving no intervention.

21 Study procedures

This clinical trial encompasses a series of laboratory studies, designed to determine which of a number of commercially available repellents provide protection against laboratory-reared *Musca sorbens* fly contact, followed by a field trial testing the protection afforded by these repellents and wearable repellent devices, from fly contact by wild *M. sorbens* on children aged three to 12 years. In the field trials, the acceptability of these products to the end-users (both children and their caregivers) will be assessed.

Prior to both trials, preliminary benchmarking laboratory studies will be conducted to determine which of five repellent actives will be studied in the clinical trials.

21.1 Laboratory trials

21.1.1 Test insects

On the day before each test, *Musca sorbens* flies of between one and 14 days post-emergence will be brought into testing room and allowed to acclimatise overnight and for at least 12 hours. Flies will be starved of their sugar and protein source (milk or milk powder) during this time period.

21.1.2 Testing room

The temperature and humidity in the room will be monitored and recorded for the duration of the study. Room temperature will be maintained at 20-27°C, and relative humidity (RH) at 20-50 %, however, it has been noted in insect rearing that *Musca sorbens* are minimally affected by changes in temperature and humidity. All tests will be conducted in the diel phase between 09:00 and 17:00.

21.1.3 Topical repellents

21.1.3.1 Protective Efficacy

Consenting participants will be asked to avoid the use of fragranced cosmetic or washing products for 12 hours prior to each laboratory trial. Immediately before testing, the participant's arm will be washed with unscented soap, rinsed with water, rinsed with 70 % ethanol in water, and towel dried. An analytical standard of the repellent will be tested at five incrementally increasing doses up to a maximum of 20 %, each diluted in ethanol.

For the first test, the diluent alone (1.4 ml) will be tested as a control. This will be applied to the participant's arm and allowed to dry for one minute. The participant will then insert his/her arm into a purpose-designed insect cage, with a hole in the top allowing a camera lens to film the upper surface of the hand and arm. The cage will contain 100 test insects, and insect behaviour on the arm will then be filmed for ten minutes. This video footage will retrospectively be analysed for the number of fly contact, and the total duration of fly contact. The participant will be instructed to refrain from moving his/her arm, which will disturb landing flies.

For the test to proceed, there must be five or more fly contacts, with the diluent (control,) in the ten-minute observation period. After this, the participant will remove their arm from the cage, carefully brushing off any flies as they leave. The lowest dose of repellent in ethanol (1.4 ml) will then be applied to the arm and allowed to dry for one minute. The participant will then re-insert his/her arm into the cage with the test insects, and insect behaviour on the arm will again be filmed for ten minutes.

This procedure will be repeated for each incremental dose of the repellent, up to a maximum of 20 % active ingredient. Each dose will be tested serially and without delay. To determine the repellent dose, the doses applied to reach that which was effectively repellent will be summed. If at any point the fly-arm contact rate drops below five in 10 minutes, the test will be stopped. After all repellent doses have been tested, 1.4 ml of the diluent control will be applied to the participant's other arm, and tested again as per the first ten-minute test, in order to verify continued fly contact/landings. If at this point there are less than five fly contacts, the results of the experiment will be discarded.

For a repellent product to be carried forward to the next phase of testing, a protective efficacy of 30 % is required. While 50 % protection is often used as a benchmark for repellent testing, the total proportion of *Musca sorbens* flies in endemic areas and carrying Ct has previously been estimated as 15 % (29). Therefore, it is plausible that reductions in fly-eye contacts of less than 50 % could still have a significant effect on the transmission capability of this vector.

21.1.3.2 Persistence

Having established which repellent products are effective at which doses, the persistence of all repellent products at doses achieving at least 30 % protection (at time zero) will be determined. Persistence will be determined using an extended version of the protocol described in 21.1.3.1. Preparation, and control testing, will be conducted in the same manner, then the repellent products at the appropriate dose will be applied and tested for ten minutes as previously, and the observation will be repeated every hour for six hours (Figure 5). After testing, the participant will be given access to washing facilities to wash off the topical repellent. This data will allow calculation of the Median Protection Time/Effective Dose (ET_{50} and ED_{50}). To determine the Median Complete Protection time (mCPT), the same protocol will be used however the first test sampling period will be extended until the first fly-arm contact.

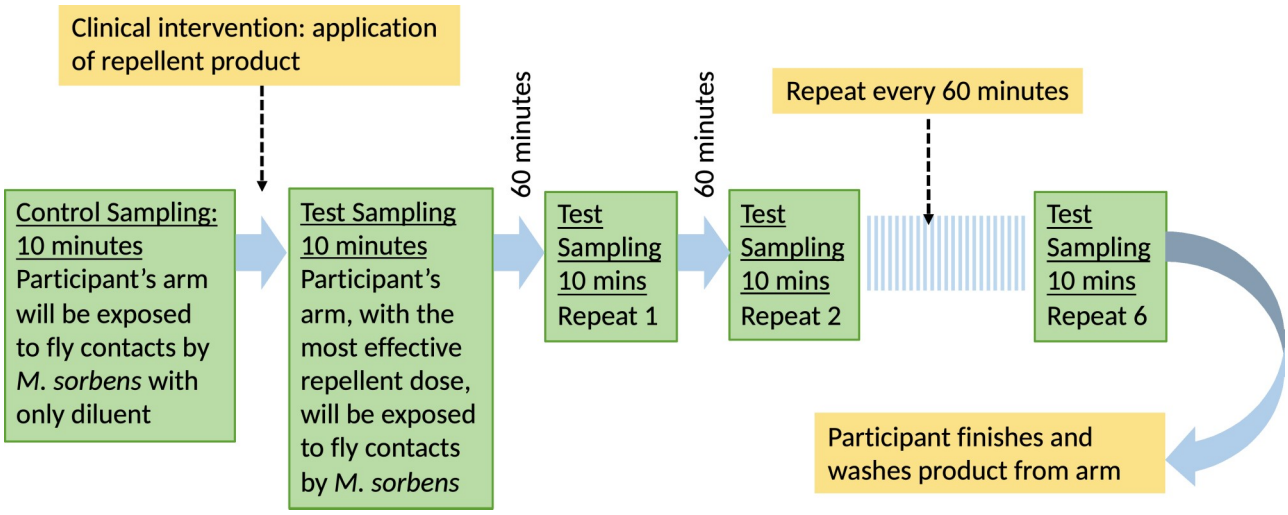

Figure 5. Timescale for repellency complete protection time (CPT), and median protection time, testing in the laboratory.

21.1.4 Wearable repellent devices

The protection afforded by wearable repellent devices against *M. sorbens* contacts, formulated at the effective dose determined in 21.1.3.1, will be measured (protective efficacy) and the persistence of this effect determined. Wearable repellent devices will be tested in an extended version of the protocol described in 21.1.3.2. Preparation and control testing will be conducted in the same manner but without the application of any diluent prior to the control test. The participant will then be given the wearable repellent device and asked to put it on (i.e. wear the necklace or neckband around their wrist), and a ten-minute test period will be conducted as previously described (21.1.3.1). After this, the observation will be repeated every hour for six hours. These procedures (control then tests over six hours) will be repeated every week for four weeks, and each timepoint participants will wear the repellent devices that they wore in the initial test at the first timepoint (Figure 6).

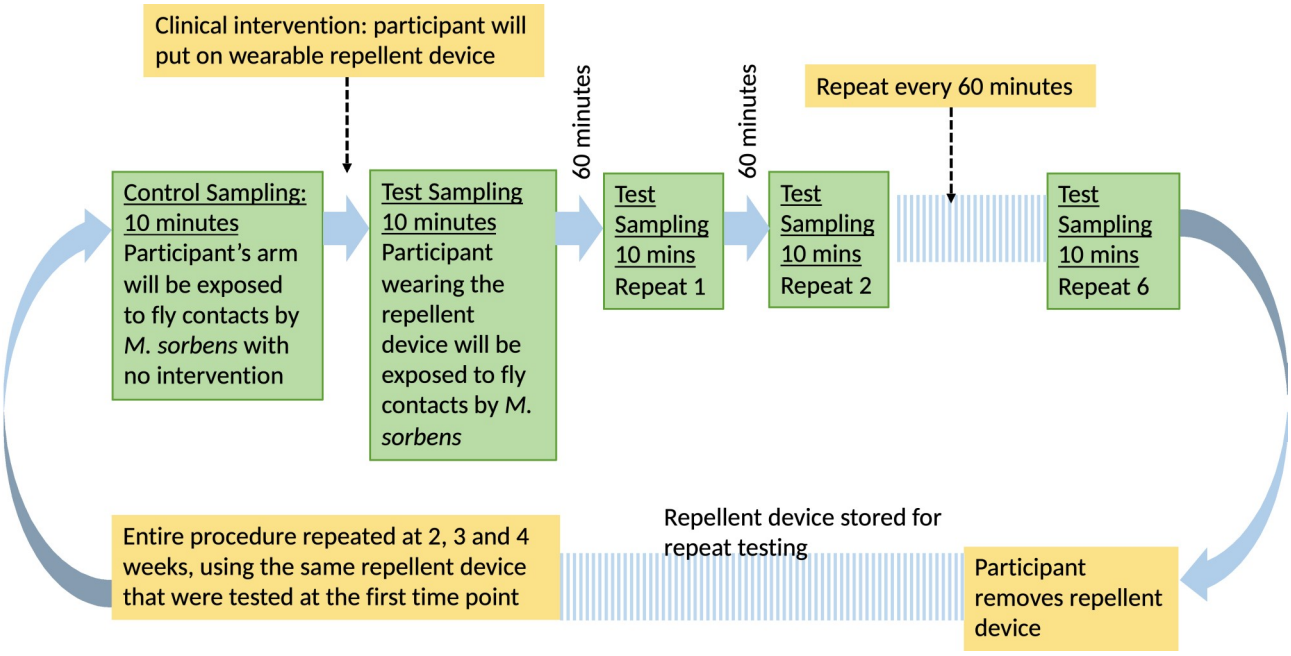

Figure 6. Timescale for wearable repellent device testing in the laboratory.

21.2 Field trials

21.2.1 Test insects and testing site

Participants will be exposed to fly-face (including eye, nose and mouth) contacts outside their house. Previous studies indicate that the majority of such contacts will be *Musca sorbens*, although *Musca domestica* may be present (46); data thus far from our field site corroborates this prediction. Ambient temperature and humidity will be recorded at every fly-face observation period. Testing will be started between 09:00 and 10:00, as preliminary data from the same locality indicate that *M. sorbens* are less active earlier in the day, allowing for the six-hour testing duration. Should the fly density at the time of year of testing be very high, the test durations may be shortened (e.g. to five minutes), as a robust measure of protection will be obtained in a shorter interval.

21.2.2 Pilot Phase

We will conduct an initial pilot study to refine and validate the proposed methodologies for Phase 2 Repellent Testing. The pilot study will focus on 10 households each with one child aged three to 12 years, on whom fly-eye observations will be conducted both before and after the application of diluent only. Semi-structured pilot acceptability interviews will be conducted, to help refine and revise the topic guides. The Pilot will further enable us to determine whether fly density in the area is appropriate to the study.

21.2.3 Topical repellents

Eligible participants will be randomly allocated in equal proportions to the study groups, as per section 13. The repellent products and product formulations to be tested will be determined before the study commences. Each study participant will test only one product type, to minimise participant discomfort. Participants will be advised not to apply any cosmetics associated with a strong scent, such as perfume, hand cream, body wash, or other scented products. Additionally, volunteers will be asked not to consume spicy foods, i.e. curries, chillies and garlic for the 12 hours prior to the tests. This will be verified with the participants prior to the commencement of any tests.

The participants face will first be washed with unscented soap then rinsed with water and towel dried. The participant will then be seated comfortably on a chair outside their house, facing the investigators (entomological field worker and nurse), and will be instructed to refrain from touching the face or brushing away landing flies. For the first test, the diluent alone (50 μL) will be tested as a control. This will be applied to the participant's face (cheeks) and allowed to dry for one minute. The participant's face will then be videoed for ten minutes by the investigator or field laboratory assistant. During this time period, fly behaviour will be manually recorded and scored. The video footage will retrospectively be analysed for (i) fly-eye (ii) fly-nose (iii) fly-mouth and (iv) fly-face contact. After this ten-minute period of control measurements, the pre-allocated topical repellent will be applied to a circular area on the participant's cheek by the Stronger-SAFE field

team nurses. Topical repellents will be applied in a 6 cm diameter circle on the cheek, at least 3 cm below the eye and positioned away from the nose and mouth. The repellent will be allowed to dry for one minute, then the videoing and observation will be repeated for another ten-minute period. Test measurements will then be repeated hourly for five further time points (Figure 7). After the final time point, the participant will be provided with soap and water to clean off the topical repellent.

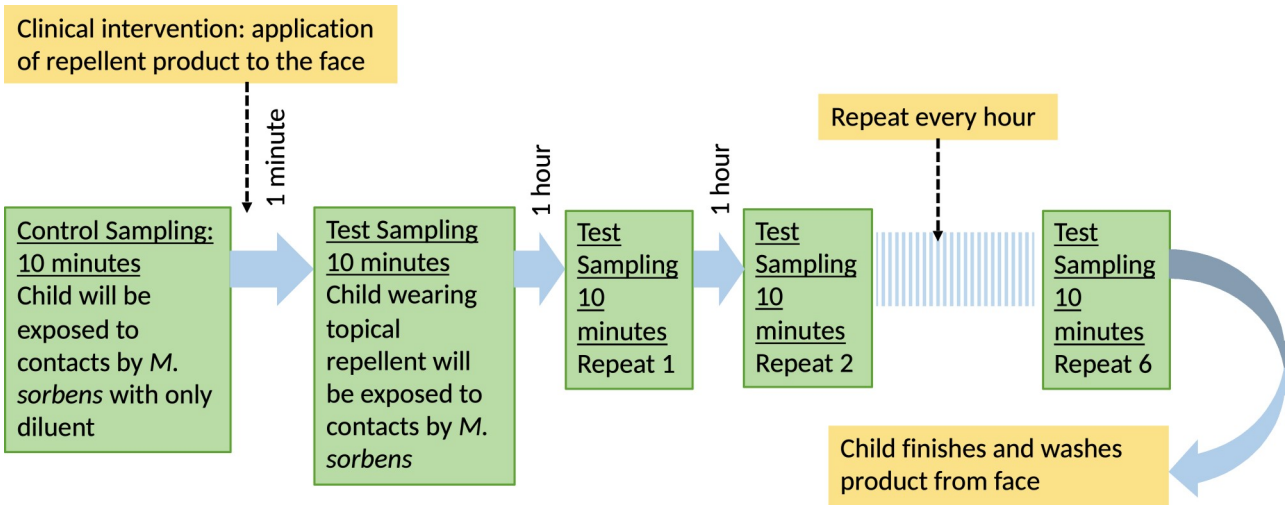

Figure 7. Study design for estimation of personal protection by topical repellents from fly-eye and fly-face contacts by *Musca sorbens*. This process will be repeated for each of 29 participants.

21.2.4 Wearable repellent devices

Control measurements will be taken for a ten-minute period, exactly as with the topical repellent testing, but omitting the face washing step. After this, the pre-allocated wearable repellent device will immediately be administered. Field nurses will demonstrate how to wear the repellent device, which will then be given to the participant to wear, or his/her primary caregiver to put on to the participant. The participant will then be seated again, and the videoing and observation repeated for another ten-minute period, as with the topical repellent testing. Again, the ten-minute observation period will then be repeated hourly for five further time points. After the six-hour test period, the participant will be asked to continue to wear the repellent device. The investigators will return one week later to repeat test sampling, at which point they will measure for ten minutes with the wearable repellent device still in place, then remove the device and measure for a further (control) ten minutes (Figure 8).

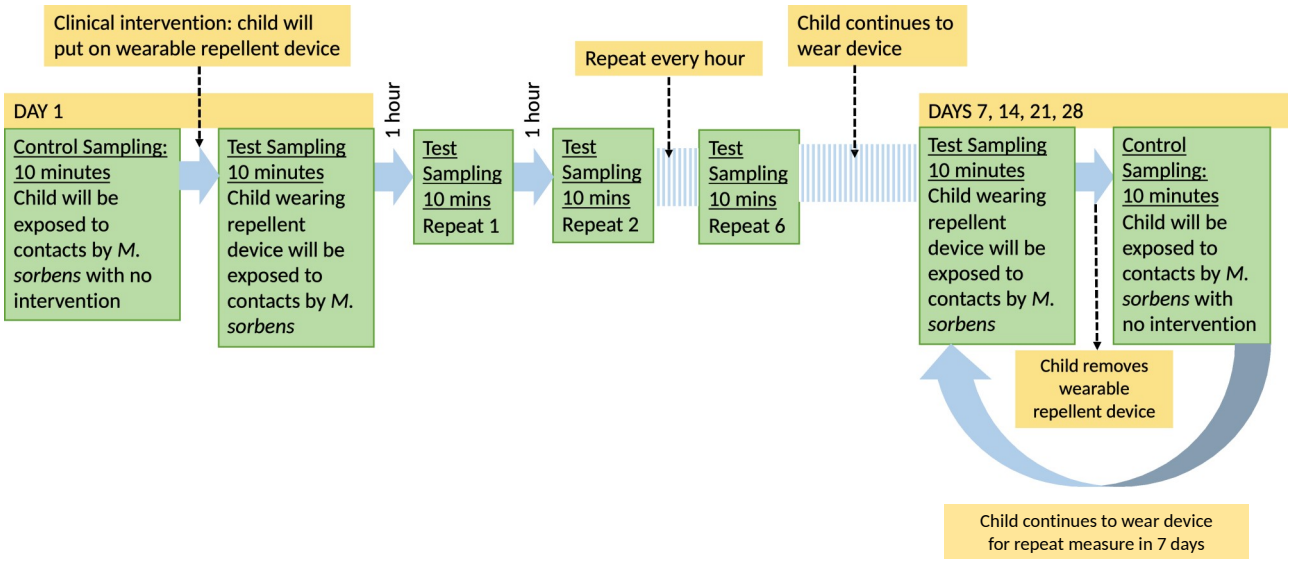

Figure 8. Study design for estimation of personal protection by wearable repellent devices from fly-eye contacts by *Musca sorbens*. This process will be repeated for each of 29 participants.

21.2.5 Acceptability *Musca sorbens* repellents

Semi-structured interviews will be conducted with all participants over 5 years of age and their primary caregivers on completion of the trial. Interviews will be short (estimated 3 -15 minutes) and, for children, will use age-appropriate questions. The purpose of the interviews is to identify major barriers to adherence to the study protocol and/or to future use of the repellents within or outside of a trial setting. The findings may also point to issues for further exploration in a more naturalistic setting. Indicative interview guides are given in Appendix 8 (Semi-structured interview_Field), and these will be refined and revised during the pilot.

For participants assigned to use the wearable devices, at the start of each data collection visit we will ask whether there have been any difficulties that prevented consistent use and whether any difficulties are foreseen. This will be done in addition to the final interview. Adherence to protocol (continued wearing of the device) will be encouraged according to section 24.

22 Risks, benefits and burden

22.1 Risk from eye-seeking flies, *Musca sorb*ens

22.1.1 Laboratory trials

Participants will be exposed to laboratory-reared populations of *Musca sorbens*. These flies have been reared in captivity for over six generations and carry no risk of Ct transmission. Participants will only be exposed to fly contact on their arms, and after completion of testing, will immediately be instructed to wash their arm. Therefore, the modified arm-in-cage assay presents only negligible risk.

22.1.2 Field trials

Participants will be exposed to natural populations of eye-seeking flies, primarily *Musca sorbens*. Because we will study the protective efficacy of these insect repellents outside the participant's houses, they will not be exposed to any greater risk than that which they experience day-to-day. However, participants will be asked to remain seated and not brush away flies from their eyes during observation periods. Previous observations in the study setting indicate that not brushing away flies is normal behaviour. Further, field work will be conducted in part by the Stronger-SAFE team ophthalmic nurses. During recruitment, children will be screened for trachoma and their trachoma status recorded. After participation in the trial is complete, all children with trachoma will be treated with Azithromycin.

22.2 Risk from insect repellent products

Up to six active ingredients (repellent products and a repellent synergist) may be used in this study, the selection of which will be made following laboratory studies that will be conducted in an insect testing facility at LSHTM, London, using laboratory-reared *Musca sorbens*. These are:

- DEET (*N*,*N*-Diethyl-meta-toluamide)
- IR3535 (3-[N-butyl-N-acetyl]-aminopropionic acid ethyl ester)
- Picaridin (2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester)
- PMD (para-Menthane-3,8-diol)
- Permethrin ((m-Phenoxybenzyl)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate)
- Vanillin (4-Hydroxy-3-methoxybenzaldehyde)

A full assessment of the risk associated with the use of the above repellents is given in sections 22.2.1 to 22.2.6 below. Here, particular attention is given to adverse incidents and events associated with repellents in the eye or on the face. Safety information regarding the repellent active ingredients used in the trial have been assessed, material safety data sheets (MSDS) and labels have been read to be sure they are safe for human use. Participants will be given an information sheet explaining the details of the ingredients and what to do if they have a reaction to the product after completion of the test. Repellent products will not be applied to broken skin.

General risks to participants associated with involvement in this study will be addressed by adhering to ICH GCP (58), the Declaration of Helsinki (59), the Data Protection Act (60) and all applicable regulatory requirements. There will be no benefit to participants. The results of this study will be used to design the trachoma transmission-blocking intervention that will be rolled out in Stronger-SAFE Phase 3, a cluster-randomised controlled trial.

22.2.1 DEET

N,N-Diethyl-meta-toluamide (DEET) is a colourless liquid that is the most common active ingredient in insect repellents. It may cause eye and skin irritation and may be harmful if swallowed.

Specifically with regards to the use of DEET on children, this repellent should not be applied to children under two years of age, further, this product should not be applied to the hands of

children under 12 years of age (61). This is to reduce the risk of ingestion by hand to mouth behaviour. It is recommended that DEET should not be applied near the eyes and mouth, children should not be allowed to handle the product and when applying on children, it should first be applied to other hands and then put on the child (62).

DEET has been found to cause serious eye effects in rabbits, where eye irritation and corneal opacity were observed but both cleared by day three and seven respectively (62). The same document noted that LD50 values in this study were “quite high”, with four grams of test material being applied per kilogram of body weight. With reference to this trial, 18.85 mg of active ingredient would be applied to the cheeks at the highest dose, 20 %, which is 0.47 % of four grams. For a child of body weight 10 Kg this would equate to 4.59 % of the AEL (Appendix 9). The same guidelines stated “If used on the face, spray on hands first and then apply sparingly and avoid eyes. Do not spray directly onto face.”, indicating acceptability of use on the face. In 2010, the European commission conducted a risk assessment to human health for DEET. They found that an R statement of R36 (irritating to eyes) was not warranted, however, because of the scores for corneal opacity in rabbits, they gave a GHS (Globally Harmonized System of Classification and Labelling of Chemicals) Category 2 (Figure 9), with an H statement of H319 (Causes serious eye irritation).

OPP Criteria, Signal Words, Symbol, and Hazard Statements	GHS Criteria, Signal Word, Pictograms, and Label Statements
PRIMARY EYE IRRITATION **Category I** Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days. DANGER No symbol Corrosive. Causes irreversible eye damage. **Category II** Corneal involvement or irritation clearing in 8-21 days. WARNING No symbol Causes substantial but temporary eye injury. **Category III** Corneal involvement or irritation clearing in 7 days or less. CAUTION No symbol Causes moderate eye irritation. **Category IV** Minimal effects clearning in less than 24 hours. No signal word, symbol or hazard statement required. Registrant may choose to use Category III statement.	**SERIOUS EYE DAMAGE/EYE IRRITATION** **Category 1** Effects on the cornea, iris or conjunctiva that are not expected to reverse or that have not fully reversed within 21 days. DANGER Corrosion symbol in diamond. Causes severe eye damage. **Category 2A** Effects on the cornea, iris or conjunctiva that fully reverse within 21 days. WARNING Exclamation mark in diamond. Causes severe eye irritation. **Category 2B** Effects on the cornea, iris or conjunctiva that fully reverse within 7 days. WARNING No symbol Causes eye irritation.

Figure 9. A comparison of chemical hazard classification and labeling: Office of Pesticide Programs (OPP) and the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (63). DEET was found to be in GHS category 2 (level unspecified) (61).

DEET has caused adverse reproductive and fetal effects in animals, and may cause central nervous system effects. There are no known carcinogenic chemicals in this product. The safety of daily application of (DEET) in the second and third trimesters of pregnancy was assessed as part of a double-blind, randomized, therapeutic trial of insect repellents for the prevention of malaria in pregnancy. The results of the study suggest that the risk of DEET accumulating in the fetus is low and that DEET is safe to use in later pregnancy (64).

By having the ophthalmic nurse apply the DEET directly to the children's face, we will avoid DEET application to the children's hands. It is possible that the child may, over the course of the day's testing, touch their face and then their mouth. However, DEET is commercially available for use on children above the age of two, despite the recommendation that it is not applied to their hands. From this, we can assume that the risk presented from skin (e.g. elsewhere on the child), to hand, to mouth is negligible, presumably because the amount of DEET that is transferred in this way is very small. Further to that, the amount that will be applied in this trial (a circle of 6 cm diameter)

will overall be much smaller than that which would be applied to the child's whole body, for example to repel mosquitoes, which it is frequently used for. Finally, for topical repellent testing, the ophthalmic nurse and team will be present at the house and near the participant all day, and will try to dissuade the child from this type of behaviour.

Current European Union guidelines concerning the risk to consumers for the use of DEET sets the $AEL_{repeated}$ (acceptable exposure level for repeated use) at 8.2 mg/kg body weight (bw)/day (61). For an estimation of the percentage of this AEL that will be topically applied of active ingredient, for a hypothetical dose range of maximum 20 % and a range of hypothetical children's body weights, see Appendix 9, for the material safety data sheet (MSDS) see Appendix 10.

22.2.2 IR3535

Insect repellent IR3535 is a liquid containing 98 % active ingredient 3-[N-butyl-N-acetyl]-aminopropionic acid ethyl ester, and 2 % inert ingredients (65). This insect repellent is structurally similar to naturally occurring beta-alanine, and is itself a substituted beta amino acid. It has been used to repel mosquitoes, deer ticks, lice and biting flies (65). At initial assessment, IR3535 was found to have low acute toxicity, and with no reports of adverse health effects on humans. However, IR3535 has been found to cause conjunctival irritation at concentrations of 10, 15 and 20 % in rabbits, further, some corneal opacities were observed which recovered in 8-21 days (66). At this time (2001) the LD_{50} given was >14,000 mg/kg orally and >10mg/kg dermally (66). In 2014 the US EPA initiated review of IR3535. They found that all human health assessment data requirements had been addressed, and between 1991 and 2014 they found 211 reports of adverse incidents in humans, which included reports of running nose and eyes and eye irritation (67). Following the review, subsequent fact sheets and technical documents state that there is reliable data regarding IR3535 to support the conclusion that this insect repellent is practically non-toxic to mammals, including infants and children (65). They found no threshold effect and therefore did not publish a margin of safe exposure. However, it was noted that eye irritation can occur if IR3535 enters the eyes (68).

Current European Union guidelines concerning the risk to consumers for the use of IR3535 sets the $AOEL_{short\ term}$ (Acceptable operator exposure level for short term use) at 6 mg/kg bw/day (69). For an estimation of the percentage of this AOEL that will be topically applied of active ingredient, for a hypothetical dose range of maximum 20 % and a range of hypothetical children's body weights, see Appendix 9, for the material safety data sheet (MSDS) see Appendix 11.

22.2.3 Picaridin

Picaridin, also known as icaridin, is a synthetic compound designed to resemble the natural compound piperine, which is found in the group of plants that are used to produce black pepper (70). It was first reviewed for toxicity by the WHO in 2001, and found to have a good safety profile with negligible dermal and limited ocular irritation capacity in rabbits (this was based on a summary of toxicity studies provided by Bayer AG, Germany) (66). The recommended target dose was 0.3 mg active ingredient/cm^2 of skin. In 2004 the WHO published the results of OECD test number 405 (Acute eye irritation/corrosion) and found Icaridin to be a slight irritant. In 2014, the US EPA reviewed the use of Picaridin, to determine whether it met the federal insecticide, fungicide and rodenticide act (FIFRA). At that time, Picaridin was second only to DEET in use in the US (71). Previously reported oral and dermal toxicological effects were determined to be species-

specific (conducted in rodents) and not relevant to humans. In a review of human incidents, 214 minor incidents were reported between 2009 and 2014, which usually involved skin, eye or respiratory irritation. Importantly, the incidents were all of minor severity and resolved rapidly. In another database (Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health (CDC/NIOSH)), 22 cases were reported between 1998 and 2010. Most cases (18 of the 19 involving picaridin alone) were low in severity, largely involving dermal or eye irritation, however, in one case where picaridin was directly applied to the face of an infant, the case was moderate in severity. The US EPA identified no human health risks associated with the use of Picaridin. Most recently, Picaridin has been classified by the US EPA as being of low acute oral, dermal and inhalation toxicity, and Toxicity Category III for primary eye and skin irritation (Category III = slightly toxic. Toxicity category not specified but assumed to be OPP, Figure 9) (72). The toxicology database is considered complete and no additional studies are required. The US EPA stated that they believe that the normal use of Picaridin does not present a health concern to the general U.S. population (72). The acute dermal LD_{50} given was >2000 mg/kg (Limit test).

Current European Union guidelines concerning the risk to consumers for the use of Icaridin sets the $AEL_{short\ term}$ (Acceptable exposure level for short term use) at 3.1 mg/kg bw/day (73). For an estimation of the percentage of this AEL that will be topically applied of active ingredient, for a hypothetical dose range of maximum 20 % and a range of hypothetical children's body weights, see Appendix 9, for the material safety data sheet (MSDS) see Appendix 12.

22.2.4 PMD

PMD, structurally similar to menthol, can be derived from the essential oil of the leaves of *Corymbia citriodora*, or from the synthetic citronellal. When *C. citriodora* oil is refined to increase the PMD content, it is known as Oil of Lemon Eucalyptus (OLE). PMD has been on the market as an insect repellent since 1998 (74), and is considered to be an eye irritant (Toxicity Category 1), and this is the only adverse effect that has been found in studies using lab animals (57). These adverse effects have led to precautions around the use of PMD, crucially, it is advised against the use of PMD on the face or hands of children (57). For this reason, PMD will not be assessed for use as a topical repellent in this clinical trial. However, the use of PMD in a wearable repellent device would be permitted, as (1) the device would not be worn on the face, and (2) exposure from a wearable repellent device is much lower as there is not direct dermal application.

Other than eye irritation, PMD is not expected to pose any health risks to people, including children (57), and no AEL/AOEL are stated. Further, due to the avoidance of PMD as a topical repellent, such values would not be relevant here. For the material safety data sheet (MSDS) see Appendix 13.

22.2.5 Permethrin

Permethrin, a member of the pyrethroid class of insecticides (75), is classed as a repellent when used to pre-treat clothing, and is the only insect repellent that is currently used for factory treatment of clothing (76). As with PMD, in this study Permethrin will only be considered for use in the wearable repellent devices.

In 2009, the US EPA evaluated multiple scenarios for permethrin factory-treated clothing, including toddlers wearing or mouthing the clothing, and found the product unlikely to pose any

significant immediate or long-term hazard to those wearing the clothing (76). Multiple studies have demonstrated that maximum permethrin uptake from impregnated clothing is five times lower than the regulations established by the WHO and US EPA, and therefore have concluded that health impairments are unlikely (77–79). This study will focus on the use of polymer coated fabric, which has the lowest skin absorption rate when compared to other types of treated clothing (39). The majority of studies also show that impregnated clothing is usually comfortable, non-irritating and non-odorous (37).

When used at appropriate concentrations, insecticide-treated clothing is deemed safe. For example, one study showed that permethrin is transferred from clothing to skin at a rate of 0.49 % per day from fabric treated with permethrin at a rate of 0.125 mg/cm^2. This equated to 0.00006 mg/kg bw/day, with the permethrin AEL $_{\text{medium term}}$ (Acceptable exposure level for medium term use) 0.05 mg/kg bw/day (80). For the material safety data sheet (MSDS) see Appendix 14.

In summary, the amount of permethrin that is allowed in treated fabric is low, and from the fabric it is thought to be poorly absorbed through the skin.

The following international agencies recommend insecticide treated apparel:

- The U.S. Centre for Disease Control and Prevention (CDC)
- The World Health Organization (WHO)
- National Institute for Occupational Health and Safety
- The American Academy of Family Physicians
- The Public Health Agency of Canada

22.2.6 Vanillin

Vanillin is derived from extract of the vanilla bean. Some studies have indicated that this compound can increase the protection time of insect repellents (81). Because vanillin is considered to cause serious eye irritation, the use of vanillin in this trial would be restricted to use in the wearable repellent technologies, which would not be proximate to the eye. For the MSDS see Appendix 15.

22.2.7 Specific risk from application of topical repellents to the face (field trial only)

Due to the nature of the eye-seeking vector in question, it is unavoidable that topical repellents must be applied to the face. As per section 16.1, the repellent active ingredients that will be tested topically (DEET, IR3535 and Picaridin) have been chosen because they do not carry a specific risk of eye irritation beyond what would reasonably be expected through application of a chemical product.

It should be noted that commercially available repellent products do have instructions on the label that recommend application to the face. However, care must be taken when doing so. In the field trial, topical repellents will be applied in a 6 cm diameter circle on the cheek, at least 3 cm (measured by three finger-breadths) below the eye and positioned away from the nose and mouth. The repellents will be administered by Stronger-SAFE ophthalmic nurses, who will be present at all times during field testing, see section 28 for more information regarding participant

care during adverse events. The importance of preventing inadvertent transfer of topical repellent from the face to the eye will be fully communicated to participants and their caregivers.

22.3 Risk from wearable repellent technologies

Wearable repellent technologies will be formulated to contain insect repellent at doses within published limit of safe application. Because the repellent product will be formulated into plastic, most likely low density polyethylene (LDPE) or high density polyethylene (HDPE), the amount of product that is released onto the skin will be considerably lower than that which is experienced via topical application of a cream.

22.4 Burden associated with participation

22.4.1 Laboratory trials

Participants will be required to make repeat visits to the LSHTM testing facility, to test each product and product formulation. During visits, they will be required to sit still for ten-minute observation periods, allowing flies to crawl freely over their forearm and hand.

22.4.2 Field trials

The participant's face will be observed and filmed for ten-minute periods. During this time, the participant will be required to sit motionless and allow flies to crawl freely over their face and around their eyes, nose and mouth. In this study setting this burden would be considered 'the norm', with children rarely bothering to brush away flies due to their extreme persistence and prevalence.

At the first time-point of testing, the child will be asked to sit for ten minutes of control measurements, immediately followed by ten minutes of test measurements, with the application of insect repellent in-between. Ten-minute duration follow-up sessions will be conducted hourly for the rest of the day (six hours). As participants may be as young as three years old, sitting motionless for these periods may be difficult, additionally, the child may become distressed.

We will encourage the children to conform to protocol as far as possible but will make reasonable judgement around levels of distress. To be, and remain, eligible for the field trial, we state "15. The participant does not become unacceptably upset during the procedures". The same methodology 'Fly-eye studies' are currently underway as part of the Stronger-SAFE Phase 1 study of Ct transmission in the same study site. In Phase 1, the team are conducting 'Fly-eye studies' on two children in each of more than 200 households, and as such have gained a great amount of experience in this type of work. The Phase 1 'Fly-eye studies' constitute two components, the ten-minute observation/videoing period as described above in this protocol, followed by 15 minutes of catching flies from the child's face. So far, we have not observed many instances in which the child becomes upset in the initial ten-minute period, with the latter fly-catching exercise generally proving more difficult. In instances where the child does become upset, the field team tries to calm the child with the help of the primary caregiver, or his/her friends and family, and encourage them to allow work to continue. If the child continues to be upset, sampling/participation is

discontinued for that child. In terms of managing distress, the same protocol will be followed for this study.

22.5 Benefits associated with participation

Participants in the laboratory trial will receive no benefits from participation in the trial. Participants in the field trial will have the opportunity to have their vision and eyes checked by the Stronger-SAFE project team, and will receive appropriate referral for identified problems. There are no further benefits expected for any participants.

23 Modifications

Should it be necessary in the field trials, lower dose topical repellents can be substituted to improve tolerance to the facial application (e.g. in the event of discomfort), provided those lower doses were found to provide at least 30 % protective efficacy.

24 Adherence

24.1 Trial adherence

The critical importance of both participant availability (for data collection), and sustained use of repellents, will be emphasised during recruitment, and participants and their parents/guardians be reminded of the importance of this when giving assent/consent. It is expected that this will present a specific challenge in the field clinical trials, where participants who test the wearable repellent devices will be required for weekly repeat testing of the product over a four-week period. Therefore, in the field trial, the following measures will be implemented:

- 'Adherence reminder sessions' will be conducted at each testing time point (e.g. at each visit to the household). The importance of allowing topical repellent to remain on the face (i.e. without washing off or removing in any other way), and of wearing the repellent device, for the full testing duration (six hours or seven days respectively) will be emphasised (Appendix 16, Adherence reminder sessions). This will be of primary importance to the wearable repellent device interventions, as for the topical repellent trials investigators will be present throughout the six-hour duration, and can supervise.
- In the six days between each testing session, for those households contactable by mobile phone, text messages will be sent to remind the primary caregiver to ensure the participant continues to wear the repellent device
- For the four, weekly follow-up testing sessions (wearable repellent devices only), on the day prior to testing one of the fieldworkers will visit the household to remind the participant's primary caregiver that the child should remain at home the following day for testing. During these 'priming' visits, the fieldworker will also discretely note whether the child is wearing the device or not, but will not comment on this or draw attention to their assessment.
- On days in-between those four weekly follow-up sessions, a limited number of unannounced visits by a member of the project team may take place to allow an

opportunity for problems relating to repellent use to be identified and addressed. Participants will be informed about the possibility of those visits at recruitment and during consenting.

24.2 Trial adherence assessment

Adherence to the protocol in the laboratory clinical trial will be simply recorded as participant presence/absence for follow-up testing.

Adherence to the protocol in the field clinical trial will comprise either: (1) wearing the topical repellent on their face for the full testing duration of six hours, or (2) wearing the repellent device for four consecutive weeks.

Adherence to protocol for topical repellents can be monitored and recorded by the field team, who will be present for the full six hours of testing. Adherence to the protocol for wearable repellent device use after the initial testing day (when the field team will be present) will be assessed by recording whether or not the participant is wearing the device at the time of each data collection visit, and at the time of each 'priming' visit on the day prior to testing. At each visit, participants will also be asked to report on any difficulties faced in continued use of the device.

25 Comparability of study groups (concomitant insect-repelling activities/factors)

Co-intervention bias is precluded in the laboratory trial by the trial design being within-subject.

To mitigate external influences on the outcome variables (frequency of fly contacts), participants in all trials will be asked to refrain from the use of any perfumed or scented product, including bathing products, for a 24-hour period before each testing session.

In the laboratory trial, co-intervention bias will be mitigated by asking participants to refrain from using any insect repellent products for a 48-hour period prior to any testing session.

In the field trial, co-intervention bias will be mitigated by asking participants and their families to refrain from the use of any insect-repelling activities for the duration of the trial. Previous observational work in the region has identified only one such activity, which involves scattering the leaves of *Schinus molle* trees on the floor and hanging these by the door. The leaves of this tree are considered to have anti-fly properties, although this belief is unsubstantiated.

26 Data

26.1 Data collection

26.1.1 Laboratory trials

Data to be collected are: Participant name and ID number, address, phone number and email address, confirmation of informed consent, date of birth, eligibility details, topical repellent skin test details (active ingredient, amount, 24 h assessment), visit details (date and time of visit to

testing facility, investigator conducting testing, person variables [body weight, tympanic temperature], temperature and humidity of testing room, repellent product and concentration tested, testing variables [Number of *Musca sorbens* flies, number of different sex, age of flies, video file ID] and adverse event monitoring).

All arm-in-cage observation and videoing will be conducted by the laboratory co-PI or GCP trained staff, as will application of the repellent product. All investigators involved in the trial will be trained in the study requirements and will follow standard operating procedures, to ensure each participant is studied in a uniform and reproducible manner.

26.1.2 Field trials

Data to be collected are: Participant, household and kebele name and ID number, phone number, woreda name, confirmation of informed consent, date of birth, eligibility details, topical repellent skin test details (active ingredient, amount, 24 h assessment), visit details (date and time of visit, fieldworkers conducting testing, person variables [ocular or nasal secretions, body weight, tympanic temperature], environmental conditions, repellent product and concentration tested, number of fly-eye, -nose, -mouth and -face contacts, video recording of face), adverse event monitoring and qualitative data from the end-of-trial product acceptability interviews.

All fly-eye observation and videoing will be conducted by trained entomological fieldworkers with experience in fly-eye studies. Application of the repellents will be conducted by qualified ophthalmic nurses. All fieldworkers involved in the trial will be trained in the study requirements and will follow standard operating procedures, to ensure each participant is studied in a uniform and reproducible manner.

26.2 Data management and confidentiality

All data will be protected and stored in compliance with General Data Protection Regulation (GDPR). Specifics per site are given below.

During screening, participants will view the participant screening form (PSF, see Appendix 17) as it is being completed. During screening and testing, all data collected (during both laboratory and field trials) will be recorded at the time of collection via electronic data capture using the Open Data Kit (ODK) secure data capture system provided by LSHTM http://opendatakit.lshtm.ac.uk/. The PSF and data collection forms will be created and managed in ODK, and study participants will not view the data collection forms. Automatic checks for invalid values, internal consistency and implausible responses will be programmed into ODK, and additional data validation checks will be run after data collection. ODK has an inbuilt audit trail. Encrypted data will be uploaded to a secure server at LSHTM for secure storage and analysis. Daily back-up of study data on central computers and servers, remote computers and hand-held devices will be conducted. Back-up data will be stored separately from the primary electronic storage, and video files (showing the participant's arm or face for laboratory or field trials respectively) will be stored on encrypted external hard drives, or encrypted and uploaded to LSHTM secure server.

After study completion, all the relevant study documentation will be retained in accordance with the local legislation, for a minimum period of 10 years after completion of the study. The final dataset will be archived and maintained by the UK PI. Anonymised data sets will be made publicly

available after publication, to ensure the data are available for other investigators to explore. Specific permission for this is requested in the consent form.

26.2.1 Laboratory trials

Data from the study will be managed by the LSHTM PI. Paper records (Informed consent/assent, PSF, adverse event monitoring questionnaire, adverse event record) will be stored in locked cabinets in the locked Arctec office in LSHTM. Scanned electronic back-ups of these will be encrypted and uploaded to LSHTM secure server.

26.2.2 Field trials

Data from the study will be jointly managed by the LSHTM and Stronger-SAFE team in Ethiopia, coordinated by the UK and Ethiopian PIs. Paper records (Informed consent/assent, PSI, adverse event monitoring questionnaire, adverse event record) will be stored in locked cabinets in the secure/locked Stronger-SAFE project office. Scanned electronic back-ups of these will be stored in encrypted external hard drives, kept separately in the same office.

The UK and Ethiopian PIs will be responsible for ensuring a secure and appropriate location for storage of study related documentation present at the field study site, as well as for ensuring that only members of site staff who are authorised have access to the files. The site Investigator File will be held at the project office in Shashemene. The Investigator File will at all times remain available for internal audits and/or inspections of regulatory authorities, including after completion of the project.

26.3 Data analysis

26.3.1 Protective Efficacy, *p*

The protection (protective efficacy, *p*) afforded by a repellent product will be presented as a percentage. *p* will be estimated by comparing fly-arm contact duration and fly-eye contact frequency, in laboratory and field trials respectively, after application (or wearing) of the repellent product to that during the control period.

Equation 1. Protective Efficacy, *p*

$p = 100 \times ((C - T)/C)$

Where (laboratory trials):

- C is the total duration of fly-arm contact before application of repellent (‘control’ measure), and
- T is the total duration of fly-arm contact after application of repellent (‘treatment’ measure)

Where (field trials):

- C is the frequency of fly-eye contacts before application of repellent (‘control’ measure), and

- T is the frequency of fly-eye contacts after application of repellent ('treatment' measure)

26.3.2 Median Complete Protection Time (mCPT)

Median CPT will be estimated in stage two ('persistence') laboratory trials only, for those repellents that demonstrated more than 30 % PE. The complete protection time for a specific dose will be estimated as the time elapsed until the first fly landing on the arm in each replicate, and based on repeat estimates of CPT, the mCPT will be estimated using a Kaplan–Meier function.

26.3.3 Median Effective Dose (ED_{50}) and Median Effective Time (ET_{50})

ED_{50} and ET_{50} will be calculated in stage two ('persistence') laboratory trials, however, as only one dosage level will be used in the field only ET_{50} will be estimated there.

The relationship between Protective Efficacy and repellent dose and time since treatment can be estimated using a probit-plane regression model (Equation 2). The coefficient b_1 provides an estimate of the effect of repellent dose on p, and b_2 provides the effect of the time since treatment on p.

Once these coefficients have been estimated, then we can estimate the **ED_{50}**, concentration (dose) of repellent product that affords 50 % protection from fly contacts at time zero (the time of application). This is done by setting p=0.5 and t_1=0 and then solving equation 2. We can also estimate **ET_{50}**, estimating the persistence of the protective effect of a repellent product for a given dosage, using the same method.

Equation 2. Probit-plane regression model for ED_{50} and ET_{50}

$$\ln [p / (1 - p)] = a + b_1(D_0) + b_2 t_1$$

Where:

- p is as above, estimated from
  - Total duration of fly-arm contact (laboratory trials)
  - Total frequency of fly-eye contacts (field trials)
- D_0 is the dose calculated as the natural logarithm of the dose applied (ln[dose])
- t is the time post-treatment in hours
- a, b_1 and b_2 are coefficients estimated using the probit–plane regression model

In the laboratory trials, p will be estimated by the total duration of fly-arm contact, while in the field trials p will be estimated using the total frequency of fly-eye contacts, as defined in section 26.3.1.

27 Monitoring

27.1 Data Monitoring Committee

A Data Monitoring Committee (DMC) of individuals that are independent of the study has been established. The DMC will be supplied interim analyses (notably after stage 1 and stage 2 laboratory clinical trials), which will be used to monitor progress of the trial, the safety data, and critical efficacy end points (Protective Efficacy). The DMC will be able to advise the sponsor and the trial steering committee if the trial should be modified, or in the worst-case scenario, prematurely terminated.

28 Safety reporting

28.1 Adverse events

Participants will be monitored throughout testing sessions by investigational staff (fieldworkers in the field clinical trials) for any adverse events. If any adverse events related to the repellent product are apparent at any time during the trial, testing will stop immediately. In laboratory trials, details of how to access treatment will be offered, and in field trials the participant will be assessed by the ophthalmic nurse in the field team.

Volunteers can only participate in a test a minimum of 72 hours after the screening for skin sensitivity to the repellent product, and participants with known allergies to any of the product ingredients will not be eligible to take part. Within 72 hours after testing, the participant will be contacted and asked to report any adverse events that might have occurred since the end of testing. Adverse events that occur >72 hours after the end of participation in the trial will be passively monitored.

An adverse event which is ongoing at the time of participant withdrawal or completion will be followed up until it resolves or until 30 days after the participant terminates from the study, whichever comes first.

28.2 Definitions

A trained clinician will evaluate the severity of an AE (Table 4). Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Table 4. Terms used to define adverse events

Term	Definition
Adverse Event (AE)/Adverse Reaction (AR)	Any untoward medical occurrence in a participant or study participant but which does not necessarily have a causal relationship with this treatment
Serious Adverse Event (SAE)	A serious event is any untoward medical occurrence that: • Results in death • Is life-threatening • Requires hospitalisation • Results in persistent or significant disability/incapacity • Consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences, or is an important medical event in connection with a clinical trial
Suspected Unexpected Serious Adverse Reaction (SUSAR)	Any adverse reaction that is classed as serious and is suspected to be caused by the product being tested, and is NOT consistent with the information about the product in the material safety data sheet (MSDS)

28.3 Reporting Procedures

All adverse events and serious adverse events will be reported to the appropriate regulatory authorities. Depending on the nature of the event the reporting procedures detailed below should be followed. Any questions concerning adverse event reporting should be directed to the CI in the first instance. For adverse event reporting, all data will be recorded via electronic data capture using ODK, then managed and stored as per section 26.

For all participants in both laboratory and field trials, the correct course of action for reporting adverse events is given in Appendix 18. Safety Reporting Flowchart_All. Any AEs occurring in participants in the Field Trial be reported in accordance with the expectations of specific regulatory bodies in Ethiopia, for which the correct course of action for reporting is given in Appendix 19. Safety Reporting Flowchart_Ethiopia.

28.3.1 Non serious AEs

All non-serious adverse events will be recorded in Appendix 20. Adverse Event Documentation Log_All.

All non-serious adverse events occurring in the Field Trial will additionally be recorded in Appendix 21.Adverse Event Documentation Log_Ethiopia, which will constitute a tabulated summary of all non-serious adverse events occurring during the field trial. This summary will be issued as a report to the relevant regulatory authorities (ORHB, FMOST and Ethiopia's Food, Medicine and Health Care Administration and Control Authority [FMHACA]) in accordance with their reporting requirements, every three months, or at the end of the clinical trial if sooner.

28.3.1.1 Non-serious AEs in laboratory trials

In the event of minor adverse reactions such as localised skin redness and swelling, volunteers will be directed to contact the nearby GP surgery at 20 Gower Street, London, WC1E 6DP (Tel. 020 7637 7628) or their own GP. They will also be supplied with the mobile number of the PI, via whom they can contact the Stronger-SAFE clinical team.

28.3.1.2 Non-serious AEs in field trials

In the event of minor adverse reactions such as localised skin redness and swelling or minor eye irritation, volunteers will be advised by the Stronger-SAFE team field ophthalmic nurses, amongst whom Mr Muluadam Abraham will be given specific extended training in adverse event management. The eye(s) will be rinsed cautiously with saline for several minutes, and the repellent washed off the face. For this eye irrigation, intravenous normal saline bags (1L) will be carried by all field teams at all times. Additionally, specific medication for treating allergic reactions in the field will be available in the field medical kit to be administered by the nurses if clinically indicated. Should further care be required, ophthalmic nurses will immediately escort the participant to Feya hospital (the best hospital in Shashmene), and the event will be considered a SAE. In addition, contact with the clinicians Dr Esmael Ali (based in Ethiopia), or the clinical team in London (Prof. Matthew Burton or Dr Anna Last) will be available by telephone while fieldwork is being conducted.

28.3.2 Serious AEs

Regardless of the relation of the adverse event to study participation, the event must be reported as a serious adverse event if it meets any of the definitions in section 28.2.

All SAEs will be recorded in Appendix 22.SAE Report_All. SAE reports will be submitted to Prof. Matthew Burton (CI) within 24 hours. Fatal or life-threatening SAEs that are assessed by the CI as being both related and unexpected (SUSAR) must be reported to RGIO and the LSHTM Ethics Committee within seven days. SUSARs that are not fatal or life-threatening should be reported to RGIO and the LSHTM Ethics Committee within 15 days of the CI becoming aware of the event.

All SAEs, SARs and SUSARs occurring in the Field Trial will additionally be recorded in Appendix 23.SAE Report_Ethiopia. This report will be sent to the relevant regulatory authorities (ORHB, FMOST and FMHACA) within 48 hours.

28.3.2.1 SAEs in laboratory trials

In the case of a severe reaction such as anaphylaxis or a severe skin reaction, it will be treated as an emergency and an ambulance will be called immediately by dialling 999 directly from a mobile, or 555 from an internal phone (this is the emergency line at reception who will then dial 999). In addition, one of two of the clinicians on the Stronger-SAFE team, Prof. Matthew Burton or Dr Anna Last will be called. Alternatively, a trained First Aider within the Keppel Street building will be called. Designated First Aiders are Vanessa Chen-Hussey (ext. 2015), James Logan (ext. 2008) and Cheryl Whitehorn (ext. 2344), but if they are unavailable First Aiders are contactable through internal phones by typing in 'first aid' to the internal phone book which will bring up a list of registered First Aiders.

28.3.2.2 SAEs in field trials

In the case of a severe reaction such as anaphylaxis or a severe skin or eye reaction, it will be treated as an emergency (SAE). For severe eye or skin reactions, the same rinsing and washing procedures will be followed as for 28.3.1.2 Non-serious AEs in field trials, then the participant will immediately be transported to Feya General Hospital (+251916301989 /+251911407518) by the field team car. All participating households will be within a 1-hour drive of this well-equipped private hospital in Shashemene (Field trial eligibility criteria, p.23). The field team will always include one ophthalmic nurse who can provide interim care until the car reaches the hospital and can access the advice of a clinician via the field co-PI (a direct mobile line to the clinicians Dr Esmael Ali (based in Ethiopia), Prof. Matthew Burton, or Dr Anna Last, who will be able to provide assistance remotely).

29 Ethics and dissemination

29.1 Research Ethics Approval

Approval for the laboratory clinical trials will be sought from the London School of Hygiene & Tropical Medicine Ethics Committee. Approval for the field clinical trials will be sought from the Federal Ministry of Science and Technology (FMOST), the Oromia Regional Health Bureau (ORHB) Ethics Committee and the London School of Hygiene & Tropical Medicine Ethics Committee. The Fred Hollows Foundation research review group will also review and endorse the protocol. All participants will provide written informed consent to take part in the study.

29.2 Protocol amendments

A formal amendment to the protocol will be required for any protocol amendments or modifications that may impact either on the conduct of the study or may affect participant safety (including but not restricted to changes in: study objectives, study design, participant population, sample sizes, study procedures, or significant administrative aspects). Substantive[1] amendments must be reviewed and agreed by the LSHTM ethics committee prior to implementation, and will be described in trial reports. Amendments will be communicated to all relevant parties via documented (and version controlled) amendments to protocols and standard operating

[1] 'Substantive' is here defined as a protocol amendment that can affect the safety of trial participants or the scientific validity, scope, or ethical rigour of the trial

procedures. Minor amendments that have no effect on the way that the study will be conducted will be agreed by the TSC and appropriately documented.

29.3 Consent and Assent

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered, and time allowed for consideration. Consent and Assent will be obtained under the jurisdiction of GDPR, that is, must be specific, freely given, granular (for a distinct purpose), clear, informed and unambiguous, properly documented and easily withdrawn.

29.3.1 Laboratory trials consent

Information about the study will be shared with potential participants by the laboratory co-PI or other GCP trained study staff. The same staff will be responsible for ensuring that all potential study participants fully understand the Participant and Product Information Sheet, the PSF and the consent forms, prior to formally agreeing to participate in the study.

29.3.2 Field trials consent/assent

Prior to approaching members of the communities in which we wish to work there will be initial dialogues with the community leaders and local health officials to introduce the purpose and nature of the research project. Following this, participants will be recruited by visiting households in the study site that are home to children in the correct age bracket. Information about the study will be shared with potential participants by members of the field research team, who have previous experience in the participant information and consenting processes. During the visit, participants will be provided with Information Sheets (Appendices 4 and 5), Product Information Sheet (Appendix 6) and Informed Consent and Assent forms (Appendix 7). Assent will be sought from the participant, and consent from the primary caregiver. This will be in Afaan Oromo, the regional language. This will be read to those who are unable read. After verbal explanation of the relevant sections of the Information Leaflet and having the opportunity to ask questions, informed consent will be gained and evidenced by a signature or thumbprint signature (deemed acceptable locally due to high rates of illiteracy), in the presence of the study team and independent witness.

Parents and guardians will be asked to provide Consent. Participants, all aged between three and 12 years, will be asked for Assent (Appendix 7).

29.4 Compensation

We will not pay individuals to participate in research studies.

29.5 Access to data

The Steering Committee will oversee data sharing between the two sites, with input from the Data Management Committee. The SC and DMC will both have access to project datasets, which will be

housed either in an Access database on the PI's "H" drive (laboratory trials only) or in a secure server at LSHTM.

29.6 Dissemination policy

Results from this study will be disseminated at local, national and international levels. The Stronger-SAFE investigator group is well placed to do this as it involves leaders within Ethiopia at the national and regional level, WHO and a leading implementing NGO. Many of the investigators are involved in the WHO GET2020 Alliance for the elimination of Trachoma.

At the end of the study, we will inform the Ethiopian regional and Federal health authority and the community about the findings of the study via a written report and direct verbal communication.

The findings will be shared directly with the communities that participated in the research through public meetings.

Formal reports will be written for the Ethiopian Federal and Regional health authority and the Federal Ministry of Science and Technology (FMOST). Reports will also be prepared for the Wellcome Trust and The Fred Hollows Foundation (Ethiopia and UK).

To ensure operational uptake of the findings of the studies, we intend to present these data at the annual National Trachoma Task Force and NTD Research Symposium (Ethiopia). Additionally, we will present this research at the annual Trachoma Scientific Informal Workshop prior to the WHO GET2020 Alliance meeting.

Scientific results will be published in Open Access in peer-reviewed journals and presented at relevant international conferences.

The Sensitisation/Community Liaison Team will disseminate the results of the study to the study community in community dialogues and radio broadcasts in conjunction with The Fred Hollows Foundation Ethiopia Communications Team.

Beyond this current phase of the work, the wider Stronger-SAFE programme will have a public engagement component, supported by the Wellcome Trust, to inform people about trachoma and share the outcomes of this work with the wider community in Ethiopia. Our concept for this is to involve community members to tell the story of trachoma in their community and how it can be controlled.

30 Timeline

The work outlined in this protocol is anticipated to take place over an eight-month period (Table 5).

Table 5. Proposed timeline for repellency trials, 2018

Month:	June	July	August	September	October	November	December	January
	1	2	3	4	5	6	7	8
Ethical approval (LSHTM)	x	x						
Ethical approval (Ethiopia)			x	x	x			
Benchmarking studies (non-clinical)	x	x	x					
Recruitment and prep (Laboratory trials, LSHTM)			x					
Laboratory trials (LSHTM)				x	x			
Recruitment and prep (Field trials)						x		
Field trials (Ethiopia)						x	x	x

31 Anticipated outputs

Results from this study will be disseminated at local, national and international levels. The Stronger-SAFE investigator group is well placed to do this as it involves leaders within Ethiopia at the national and regional level, WHO and a leading implementing NGO. Many of the investigators are involved in the WHO GET2020 Alliance for the elimination of Trachoma.

At the end of the study, we will inform the Ethiopian regional and Federal health authority and the community about the findings of the study via a written report and direct verbal communication. The findings will be shared directly with the communities that participated in the research through public meetings. Formal reports will be written for the Ethiopian Federal and Regional health authority and the Federal Ministry of Science and Technology (FMOST). Reports will also be prepared for the Wellcome Trust and The Fred Hollows Foundation (Ethiopia and UK).

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