ARCTEC REPELLENT TRIALS

Title: Comparison trial between two repellent products and a positive control against *Culicoides nubeculosus* midges.

arctec ref #928

Protocol Version 1.2

Date: 25th October 2018

LSHTM ethics reference: 16094

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Sponsor and Funder

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Sponsor and Funder

This protocol provides information about procedures for entering participants into *arctec* repellent trials. The protocol should not be used as a guide for the treatment of others; every care was taken in its drafting, but corrections or amendments may be necessary.

Problems relating to this trial should be referred, in the first instance, to the Trial Coordination Centre.

This trial will adhere to the principles outlined in the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines, protocol and all applicable local regulations.

Signatures

This protocol has been read and approved by:

On behalf of the Trial Coordination Centre, *arctec*

31st Aug 2018

Prof James Logan	Date
Chief Investigator	

On behalf of the Sponsor, NEO-INNOVA HEALTHCARE LIMITED

John Davies	Date
Director	

Study Synopsis

Name of Sponsor:	NEO-INNOVA HEALTHCARE LIMITED		
Title of Study:	Comparison trial between two repellent products and a positive control against *Culicoides nubeculosus* midges		
Short Title:	Midge Repellent Trial 928		
Protocol Number:	928	**Version:**	1.0
Chief Investigator:	Professor James Logan, Arthropod Control Product Test Centre (*arctec*), LSHTM		
Type of Product:	Biocide Product Type 19	**Phase of development:**	IV
Name(s) of Finished Product:	NEO-PART prolonged action release technology PMD (40% PMD) NEO-PART prolonged action release technology PMD (35% PMD) Positive control (20% Picaridin, 'Smidge')		
Primary/Secondary Objective(s):	***Primary objective:*** To determine and compare the median complete protection time (CPT) of two insect repellent products and a positive control against *Culicoides nubeculosus* (midges). ***Secondary objective:*** To determine and compare the protective efficacy of two insect repellent products and a positive control against *Culicoides nubeculosus* (midges).		
Study Design / Methodology:	A single-centre laboratory setting study using healthy volunteers to test two insect repellent products (NEO-PART prolonged action release technology PMD (40% PMD) And NEO-PART prolonged action release technology PMD (35% PMD)) and a Positive control product (20% Picaridin, 'Smidge') against *Culicoides nubeculosus* midges.		
Number of Participants:	A total of 7 volunteers will be used to determine the median Complete Protection Time and protective efficacy of two insect repellents and a positive control against one midge species.		
Intended Product Users:	The test products are designed for use by the general public in areas where midge biting is likely.		
Study Duration:	Participants will undergo a screening evaluation which includes a bite test (monitored for up to 72 hours), followed by up to 3 repellency tests of up to 12 hours duration. Each participant will be followed up after each visit by email/ in person/ by phone within 72 hours of the visit to monitor for adverse events. The minimum interval between the two repellency tests will be 72 hours. The minimum duration of participant involvement would therefore be approximately 3 weeks. Total study duration (recruitment and participant involvement) is anticipated to be 1 month.		

Inclusion Criteria:	• Able and willing to give fully informed consent; • Able to understand and comply with the study procedures; • Consider themselves to be in good general health; • Male or female; • Aged 18 to 65 years; • Non-smokers or willing to refrain for 12 hours prior to and during each test; • Willing to undergo a bite test with *Culicoides nubeculosus* midges with up to 72 hour follow-up.
Exclusion Criteria:	• Suspected or known to be sensitive or allergic to midge bites; • Participated in an interventional study (other than a biting insect challenge study) in the previous 3 months; • Participated in a biting insect challenge study in the previous 72 hours; • Aware of having any cardiovascular or respiratory disorder (whether active or inactive) (e.g. asthma); • Individuals with localized skin disorders affecting the forearm; • Allergic to any of the test product ingredients; • Extensive tattooing or other conditions of the forearm which would make interpretation of the bite test results difficult; • Women who are pregnant, nursing or intending to become pregnant; • Previous anaphylaxis; • Aware of having a compromised immune system;(an immune system that is temporarily or permanently incapable of working at full capacity); • Phobia of flying insects; • Travelled to a mosquito-borne disease endemic area in the last 3 months.
Efficacy Endpoint(s):	***Primary:*** Median Complete Protection Time for the repellent products, tested against *Culicoides nubeculosus* midges. ***Secondary:*** Percentage protective efficacy of the repellent products, tested against *Culicoides nubeculosus* midges.
Safety Endpoint(s):	Adverse event data will be collected and summarised.
Statistical Methods:	Median Complete Protection Time will be calculated using the Kaplan Meier survival-function. Two-way analysis of variance followed by Tukey's tests or general linear mode will be used to compare the mean complete-protection time for the tested repellents
Investigation Site(s):	***Single-centre:*** Arthropod Control Product Test Centre (*arctec*), Chariot Innovations Limited, a wholly-owned subsidiary of the London School of Hygiene & Tropical Medicine (LSHTM).

TABLE OF CONTENTS

1. Introduction

Mosquitoes, midges and other biting insects are vectors of extremely important diseases such as malaria, yellow fever, filariasis and many viruses and also may be of great nuisance value. The use of repellent products can provide added personal protection from disease transmission and nuisance bites. New effective repellents would offer an additional option for protection against biting insects. The tests carried out by *arctec* provide important information on the effectiveness of skin repellents, which will be used for label claims to accurately inform consumers and registration purposes.

The aim of this study is to determine the effectiveness of two topically applied insect repellent products provided by NEO-INNOVA HEALTHCARE LIMITED and one positive control containing the UK repellent brand 'Smidge'.

2. Objectives

Primary objective: To determine and compare the median complete protection time (CPT) of two insect repellent products and a positive control against *Culicoides nubeculosus* (midges).

Secondary objective: To determine and compare the protective efficacy of two insect repellent products and a positive control against *Culicoides nubeculosus* (midges).

3. Study Design

This is a single-centre, single-arm study with all participants testing two formulations containing PMD and a positive control product containing Picaridin. The control for each test is an untreated arm. There is no blinding or randomisation employed, since the outcome measures are based on midge behaviour. Repellent product testing takes place in a laboratory setting using 7 participants (preferably gender balanced). Each participant will test both PMD formulations and a positive control product (see section 8).

- *Culicoides nubeculosus* is a common species of biting midges belonging to the family Ceratopogonidae. *Culicoides* midges are small biting flies found throughout the world, breeding primarily in moist, damp, muddy habitats. To develop their eggs, female *Culicoides* require a blood meal from warm-blooded vertebrates, most often a domesticated animal and humans. *Culicoides* are implicated in the transmission of multiple zoonotic diseases, such as bluetongue virus, African horse sickness, and bovine ephemeral fever, and their bites can be painful and can cause uncomfortable irritation to both humans and livestock.

Midges will be obtained from insecticide-susceptible reference strains held at the Pirbright Institute (Institute for Animal Health). They will be maintained at 22-24°C, 80-90% relative humidity, with a 12:12 hour photoperiod.

3.1. Study Endpoints

The primary endpoint is the median Complete Protection Time (CPT) of the repellent products. The CPT is defined as the time between application of the repellent product and the occurrence of the first probe or bite in a 1 minute test, followed by a confirmatory probe or bite within 30 minutes.

The secondary endpoint is an assessment of the products Protective Efficacy (PE) over time. Protective Efficacy over time will be expressed as the percentage of midges probing or biting at hourly intervals from the time of the repellent application to the end of the test (when 50% or more midges are probing or biting the test arm vs. untreated control, or 12 hours).

3.2. Risks and Benefits

3.2.1. Midges

Participants will be exposed to insect bites and may experience some irritation and itching. The midges are reared in insectaries and have been in culture for many years and are pathogen free. In order to minimise the risk of infection the following steps have been taken:

- Midges used in the test are 3-8 days old and have never had a blood meal.
- One cage of midges is used on one volunteer only and after the test, those midges are destroyed. Therefore, no two volunteers are ever bitten by the same midges and the midges are never returned to the colony.
- There will be a minimum of 72 hours between product tests in order to minimise any possibility for sensitization to midges bites

3.2.2. Product

p-Menthane,3-8-diol (PMD) is a recommended active ingredient, which has been evaluated for safe use by the U.S. Environmental Protection Agency (USEPA)[1]. According to the USEPA laboratory exposure of animals to the active ingredient, PMD, this resulted in no adverse effects except eye irritation. The Product Safety Data Sheets for the PMD product classify PMD as seriously irritating to eyes. The eye irritation potential of the finished product formulation has not been studied; therefore as a precaution, the products will be handled in the same way as the active ingredient. The researcher and participant must wear eye protection during application of the product and the potential for eye irritation is described in the Participant Information Sheet. Correct handling of the product by the researcher according to ARC-SOP-006 “Safe Handling and Application of Insect Repellent PMD” will avoid risks associated with the eyes. Participants will be excluded if they have a known allergy to any of the product ingredients, or any skin condition, which may affect their reaction to the product.

The active ingredient, Picaridin, has been classified as potentially mildly irritating/uncomfortable to the eyes – participants and researcher will wear safety glasses during handling of the products and the participants will be warned about the potential for irritation during the Informed Consent Procedure. Participants will also be excluded if they have a known allergy to any of the product ingredients, or any skin condition which may affect their reaction to the product.

Specifically, for both products, the staff member should:

- Handle and apply small volume of product that was beforehand calculated for each participant
- Apply the products in a well ventilated room (air extractors and vents are available and can be turned on in the laboratory when the product is applied) to avoid risks associated with potential respiratory track irritations
- Ensure that the eyes of the staff member and the participants are protected with safety glasses when the products are being measured and applied;
- Remind the participant not to transfer the repellent from their arm to their face, either directly, or by touching their arm and then their face. Gloves are required during testing;
- Rinse with clean water if the eyes come into contact with the repellent, and seek medical advice from a first-aider (dial 0 to ask reception or look up 'First aid' in the LSHTM Telephone Book)
- There will be a minimum of 72 hours between product tests in order to minimise any possibility for sensitization to products over time.
- Report any adverse events

There is no evidence to suggest that the active ingredients present any risk to pregnant or lactating women or the unborn child. However, since participants will be exposed to and may experience increased sensitivity to midge bites, pregnant women will not be included in the study.

General risks to participants associated with involvement in this study will be addressed by adhering to ICH GCP[2], the Declaration of Helsinki[3], the Data Protection Act[4] and all applicable regulatory requirements.

There will be no benefit to participants. The results of this study will inform the products labelling.

4. Participant Entry

4.1. Screening Procedures

Volunteers will be consented prior to any screening procedures being undertaken. Volunteers who do not meet the criteria for eligibility will be excluded. In order to assess sensitivity in those volunteers who claim not to be sensitive to insect bites, volunteers will be given a bite test using the midge species used for testing. This will involve administering one *C. nubeculosus* bite and monitoring the reaction to the bite for up to 72 hours. Volunteers whose reaction (area of wheal) is larger than 1 cm, or if the participant scores their itchiness or redness as 3 out of 3 (with "1" being "no itchiness/redness", "2" being "some itchiness/redness", and "3" being "very itchy/red"), they will be unable to test with that midge species. Volunteers who fail the screening procedures will be advised that they will not proceed in the study and will be withdrawn and replaced.

4.2. Inclusion Criteria

Volunteers will be healthy individuals and chosen based on their insensitivity to the bites in order to limit any itchiness or discomfort.

Volunteers will be included in the study if they meet all of the following criteria:

- Able and willing to give fully informed consent;
- Able to understand and comply with the study procedures;
- Consider themselves to be in good general health;
- Male or female;
- Aged 18 to 65 years;
- Non-smokers or willing to refrain for 12 hours prior to and during each test;
- Willing to undergo a bite test with *C. nubeculosus* midges with up to 72 hour follow-up.

Volunteers will be advised not to apply any cosmetics associated with a strong scent, such as perfume, hand cream, body wash, or scented. Additionally, volunteers will be asked not to drink alcohol or consume spicy foods, i.e. curries, chillies and garlic and to not to engage in vigorous exercise for the 12 hours prior to the tests. This will be verified with the participants prior to the commencement of any tests.

4.3. Exclusion Criteria

Volunteers will be excluded from the study if they meet any of the following criteria:

- Suspected or known to be sensitive or allergic to midge bites;
- Participated in an interventional study (other than a biting insect challenge study) in the previous 3 months;
- Participated in a biting insect challenge study in the previous 72 hours;
- Aware of having any cardiovascular or respiratory disorder (whether active or inactive) (e.g. asthma);
- Individuals with localized skin disorders affecting the forearm;
- Allergic to any of the test product ingredients;
- Extensive tattooing or other condition of the forearm which would make interpretation of the bite test results difficult;
- Women who are pregnant, nursing or intending to become pregnant;
- Previous anaphylaxis.
- Aware of having a compromised immune system; (an immune system that is temporarily or permanently incapable of working at full capacity);
- Phobia of flying insects;
- Travelled to a mosquito-borne disease endemic area in the last 3 months.

4.4. Withdrawal Criteria

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.

5. Randomisation and Enrolment

Volunteers 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. Volunteers will be fully informed before the study and it will be made clear that they can withdraw from the study at any time. Volunteers will be given and asked to read the Participant Information Sheet and Product Information Sheet which describes the tests which they will take part in, and a consent form which must be signed before the test begins.

6. Treatments

NEO-INNOVA HEALTHCARE LIMITED will provide two products to be tested containing 35% and 40% PMD, and one positive control containing 20% Picaridin. The positive control product is currently marketed as a midge repellent, while PMD is a recommended active ingredient, which has been evaluated for safe use by the U.S. Environmental Protection Agency (USEPA)[1]. Therefore, humans have already used the products. The products will be applied at a rate of 1.67 µl/cm^2. The products will be applied to the measured forearm of each participant. This dose does not exceed the maximum daily limit for PMD or Picaridin.

7. Safety Reporting for Non-Drug Trials

7.1. Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or study participant
Serious Adverse Event (SAE)	A serious event is any untoward medical occurrence that: Results in death Is life-threatening Requires inpatient hospitalisation or prolongation of existing 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.

Medical judgement should be exercised in deciding whether an AE is serious in other situations. 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.

7.2. Reporting Procedures

All adverse events and serious adverse events should be reported. Depending on the nature of the event the reporting procedures listed below should be followed. Any questions concerning adverse event reporting should be directed to the chief investigator in the first instance.

7.2.1. Non serious AEs

All adverse events should be recorded on the Adverse Event Record Form (Appendix 1) and Adverse Event Monitoring Questionnaire (Appendix 2) and entered into a spreadsheet and stored on the "I" drive with access limited to the *arctec* study staff. All adverse events will be reported to NEO-INNOVA HEALTHCARE LIMITED. Depending on the nature of the event the reporting procedures below should be followed, see Appendix 3 for a flowchart of safety reporting.

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.

7.2.2. 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 7.1. AE questionnaires meeting the SAE definition will be

submitted to Dr James Logan within 24hours. SAEs that are assessed by the CI as being both related and unexpected must be reported to the LSHTM EC within 15 days of the CI becoming aware of the event.

In the case of a severe reaction such as anaphylaxis, 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, a trained First Aider within the Keppel Street building will be called. Designated First Aider is 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. All *arctec* staff (Robert Jones ext. 8216, Chelci Squires ext. 7939, Vanessa Chen-Hussey ext. 2015) and some of the other insectary staff (Shahida Begum ext. 2372) have also been trained in anaphylaxis.

In the event of an acute allergic reaction, a trained First Aider within the Keppel Street building will be called and the member of staff present will remove the trigger if possible. This will include removing any biting mosquitoes and washing the product off the participant's arm.

8. Test methodology

Female midges will be host-seeking, of uniform age and 3-8 days post-emergence. Active host-seeking females will be selected to ensure a good response from the test midges using an aspirator or an appropriate airflow apparatus.

Fifty female midges will be placed into a cage 20x20x20cm inside the testing room at least one hour before the test commences. Testing should be conducted in a testing room maintained at 25±2ºC and 80% RH.

The researcher will verify that the participant has adhered to the pre-testing instructions. Non-compliant participants will be unable to participate in the test on that day and will be booked in for another appointment.

8.1. Midge Fitness Check and control test (combined)

Before the start of each test, the participant will insert a bare (control) left arm into the cage for 1 minute to assess biting activity of the midges. Only cages with at least 10 midges landing within one minute will be used in the tests. This same procedure is used to assess whether volunteers are attractive to the midges. The number of midges probing or biting the arm after 1 minute will be counted and recorded (to provide biting rate data of the control test for the Protective Efficacy endpoint) and the arm removed from the cage.

8.2. Product application

The products will be applied to the forearm between the wrist and elbow. Volunteers' forearm surface area is estimated using the WHO protocol for testing skin repellents below:

$$Area = {}^{1}/_{2} \ \ (C_W + C_E)\ X\ D_{EW}$$

Where C_W is the circumference of the wrist; C_E is the circumference of the elbow; and D_{EW} is the distance between the wrist and elbow in centimetres (cm). The dose is expressed using µl/cm^2. The appropriate dose is then measured using a micropipette or balance and applied to the arm using a gloved finger.

8.3. Complete Protection Time

The repellent product will be applied to the right arm of the participant. A combined fitness check and control test is conducted as described in section 8.1. Immediately after, the right (treated) arm and inserted into the cage for 1 minute. The number of midges probing or biting the arm after 1 minute will be counted and recorded. A further hour post-application of the repellent product, the left arm (control) of the volunteer is again inserted into the cage. The number of midges probing or biting the arm after 1 minute will be counted and recorded and the arm removed from the cage. If less than 10 midges land on the untreated arm during this 1 minute test the cage will be refreshed with new midges and the test repeated. Immediately after, the right (treated) arm is again inserted into the cage for 1 minute. The number of midges probing or biting the arm after 1 minute will be counted and recorded. This procedure will be repeated at hourly intervals throughout the test.

If at any stage, more than one midge probes or bites on the treated arm in the 1 minute test, this represents the end of the testing for Complete Protection Time. If, at any stage, one midge probes or bites on the treated arm in the 1 minute test the test will be repeated half an hour later. If there is a confirmatory probe or bite during the test, this represents the end of the testing for Complete Protection Time but testing for Protective Efficacy (as described in section 8.4) will continue at hourly intervals, with the next test taking place 30 minutes later. If there is no confirmatory probe or bite during this test, testing will resume, with the next test to take place 30 minutes later. All testing for Complete Protection Time and Protective Efficacy must be conducted at hourly intervals.

8.4. Protective Efficacy

In order to determine Protective Efficacy over time, the tests will continue every hour as described in section 8.3 until at least 50% of midges are probing or biting compared with control (i.e. 50% Protective Efficacy), or until 12 hours post application.

8.5. Follow-up after testing

Participants will be followed up within 72 hours after the test to assess any possible side effects or reactions to the bites and/or products.

9. Statistics and Data Analysis

9.1. Sample size calculation

Using data available from previous arm-in-cage repellent tests using midges, the power was determined for estimation of Complete Protection Times (CPT, primary outcome) at different values and standard deviations (Appendix 4). The sample size was calculated using the sample size formula for the comparison of 2 means with equal standard deviation. The required sample size per group was given by:

$$n = \frac{2\sigma^2}{\delta^2} f(\alpha, \beta)$$

Where δ^2 is the difference between the mean CPTs of 2 treatment groups, σ is the standard deviation of both treatment groups for one comparison which are assumed to be equal. $f(\alpha, \beta) = \left(z_{1-\alpha/2} + z_{1-\beta}\right)^2$, which varied depending on the specified values of α and β.

9.2. Data analysis

The endpoint for Complete Protection Time will be time to treatment failure for each participant test. Treatment failure is the time at which the product no longer provides complete protection, which is determined as the time at which one probe or bite occurs in a 1 minute test, followed by a confirmatory probe or bite within 30 minutes. Using IBM SPSS Statistics 20, the times to treatment failure will be analysed using Kaplan-Meier Survival functions, and from these the median Complete Protection time and 95% confidence intervals will be calculated.

A linear regression will be used to compare the mean complete-protection time for the tested repellents. As a sensitivity analysis we will use linear regression to estimate the mean CPT difference between groups, but we will generate robust standard errors by relaxing the assumption of independence of observations between the same participant within the study, allowing for correlation between observations from the same participant in the variance-covariance matrix.

The Protective Efficacy over time from application will be calculated using the formula below at each hour and presented as a summary table and graph. Using IBM SPSS Statistics 20, the PE will be analysed using a two-way analysis of variance followed by Tukey's tests or generalised linear model.

$$\text{PE} = \frac{\text{Midges probing on control} - \text{Midges probing on treatment}}{\text{Midges probing on control}} \times 100$$

Adverse Events will be tabulated and included in the study report. Adverse events occurring after the end of participant participation but before the end of the study will be listed separately.

10. Safety and Data Monitoring

10.1. Risk Assessment

Using the LSHTM 'Monitoring Risk Assessment' tool, the CI has determined studies of this kind to be "low-risk". Day-to-day monitoring will be carried out at the study centre by a member of the study team with delegated responsibility.

Safety information regarding the repellent used in the trial have been assessed, material and safety data sheets (MSDS) and labels have been read to be sure they are safe for human use. The active ingredient in the repellent to be tested is PMD or Picaridin. Participants will be given an information sheet explaining the details of the ingredients and what to do if they have a reaction to the products or the midges after completion of the test.

10.2. Adverse events

Volunteers will be monitored throughout the duration of the tests by investigational staff for any adverse events. If any adverse events related to insect bites or the repellent products are apparent at any time during the trial, testing will stop immediately and details of how to access treatment will be offered. Volunteers can only participate in a test a minimum of 72 hours after the screening bite test and participants with known allergies to any of the product ingredients will not be eligible to take part.

Within 72 hours after laboratory testing an email will be sent to participants asking them 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.

10.3. Data Monitoring

With the exception of the Volunteer Questionnaire, which is completed by the participant, all data collected will be recorded in the case report forms (CRF) and signed by the person completing the CRF. The CRF is considered to be source data.

Data to be collected are: Participant number and date of visit on every page, confirmation of informed consent, date of birth, eligibility details, bite test details (midge species, timing, 10 min and 72 h assessments), test visits (eligibility checklist, forearm measurement, product details, product application details, arm-in-cage testing data (time, fitness check, No. midges probing control arm, No. midges probing treated arm)) and adverse event monitoring.

Raw data from the CRF are then entered into an Excel spreadsheet for analysis and saved on the shared "i" drive (with access limited to members of the study team). Data will be double entered and verified to ensure accuracy. CRFs will be kept in locked storage.

Information in the database for each test will be linked to a relevant SOP, risk assessment, contract, and files of statistical analysis and location of report copy.

11. Regulatory Issues

11.1. Ethics approval

arctec will obtain approval from the LSHTM Ethics Committee for this trial. *arctec* will require a copy of the ethics approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human participants adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

11.2. Consent

Consent to enter the study must be sought from each volunteer only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed volunteer consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected.

11.3. Confidentiality

Participants' identification data will be required for the enrolment process. The Trial Coordination Centre will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

11.4. Sponsor

NEO-INNOVA HEALTHCARE LIMITED

11.5. Funding

NEO-INNOVA HEALTHCARE LIMITED will fund the study. Volunteers will be paid £60 per day (12 hour testing) to compensate for their time.

11.6. Record retention

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

12. References

1. USEPA (2000) p-Menthane-3,8-diol (011550) Fact Sheet EPA. Office of Pesticide Programs, Environmental Protection Agency, NW Washington, D.C. 20460. Available online:

http://www.epa.gov/opp00001/chem_search/reg_actions/registration/fs_PC-011550_01-Apr-00.pdf [accessed 09FEB16].

2. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (1996) 'ICH Harmonised Tripartite Guideline E6(R1): Guideline for Good Clinical Practice'.
3. World Medical Association Declaration of Helsinki (1964) 'Ethical Principles for Medical Research Involving Human Subjects'.
4. Data Protection Act 1998. London

Appendix 1: Adverse Event Record Form

Study Title:

Volunteer reference number	**Date**	**Side effect/adverse event**	**Related to the product**	**Comments**	**Action taken**
			Y/N/ don't know		

Appendix 2: Adverse Event Monitoring Questionnaire

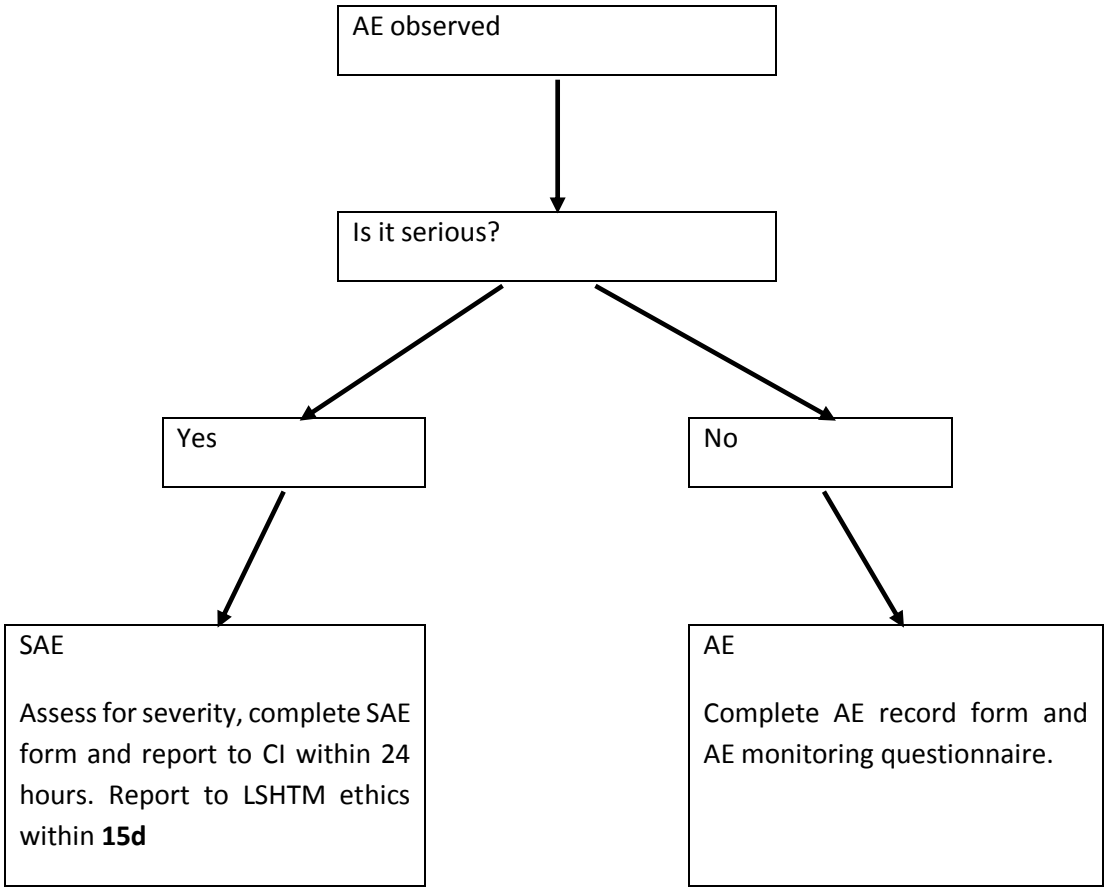
To be completed by person who experienced the Adverse Event	
Name (first name SURNAME)	
Date of birth dd/mm/yyyy	
Phone number	
Mobile number	
E-mail address	
What kind of adverse event did you experience? e.g. skin rash, burning sensation, severe allergic reaction	
How long did the adverse event last?	
How serious was the event? mild / moderate / severe / life threatening	
Did you take any action to resolve the event?	
Was any treatment required?	
Did you visit A&E? Please enter details	
Did you stay in hospital overnight? Pease enter details (no. nights/admission)	
Outcome unresolved / resolved / resolved with sequel	
To be completed by arctec trial manager	
Participant ID	
Study title	

Study code	
Type of study e.g. repellent, impregnated clothing, after-bite cream	
Exposure type e.g. chemical, mosquito bites, bed bug bites	
Exposure area e.g. forearm, legs	
Active ingredient	
Report date dd/mm/yyyy	
Other event Please enter details	
Likelihood of Adverse Event being related to study unrelated / unlikely / possible / probably / definite	
Serious Adverse Events	
Was the event serious? yes / no	
Admitted to Intensive Care Unit? yes / no	
Seriousness criteria (please tick)	
life threatening	
required hospitalisation	
prolonged hospitalisation	
congenital anomaly	

disabling/incapacitating	
important medical event	
required intervention to prevent impairment or damage	
Fatal	
If fatal, date of death dd/mm/yyyy	
Primary cause of death	
Was a post-mortem performed? yes / no	
Date adverse event become serious dd/mm/yyyy	
Possible contributing factors to SAE other than study participation or underlying disease being studied Please give details	
None apparent	
Concurrent illness, disease or other external factors	
Concurrent medication	
Study procedure	
Accident, trauma, or other external factors	
Other	
Relevant concomitant medication at time of SAE yes / no – if yes please provide details	
Treatments/procedures for SAE yes / no – if yes please provide details	

Relevant medical history (include only relevant past or concurrent medical disorder, surgeries, etc that might help explain the SAE) yes / no – if yes please provide details	
Relevant laboratory testing yes / no – if yes please provide details	
If relationship to study participation was unrelated, provide causality Please give specific details	
Discontinuation of study participation	
Concurrent disorder	
Concomitant medications	
Other	
If action taken with study participation, was study interrupted or discontinued? Provide date (dd/mm/yyyy)	
Did SAE abate after study was stopped? yes / no / not applicable / unknown	
Did SAE reoccur after reintroduction of study participation? yes / no / not applicable / unknown	
Narrative/Comments Please describe the SAE including a chronological clinical presentation and evolution of the SAE and associated signs/symptoms	
Please submit this questionnaire to Dr James Logan. SAEs that are assessed by the CI as being both related and unexpected must be reported to the LSHTM EC within 15 days of the CI becoming aware of the event.	

Appendix 3: Flowchart for Safety Reporting



Appendix 4: Sample Size Calculation for a comparison trial between a positive control and two midge repellent products

A sample size calculation was done using CPT data from a previous study of a repellent against *Culicoides nubeculosus*. The sample size was calculated using the sample size formula for the comparison of 2 means with equal standard deviation. The required sample size per group is given by:

$$n = \frac{2\sigma^2}{\delta^2} f(\alpha, \beta)$$

Where δ^2 is the difference between the mean CPTs of 2 treatment groups, σ is the standard deviation of both treatment groups for one comparison which are assumed to be equal. $f(\alpha, \beta) = \left(z_{1-\alpha/2} + z_{1-\beta}\right)^2$, which varied depending on the specified values of α and β.

Sample size calculation outcomes

The analysis revealed that for an experiment to compare mean CPT (in min) between any PMD products with Picaridin 20%, assuming a difference in mean CPT of 120 min, with a power of 80% and a significance level of 5%, 7 participants would be required.

A Bonferroni correction was applied for this analysis. The significance level at 5% (alpha = 0.05) was divided by the number of comparisons being made to maintain overall significance level (here divided by 2, as 2 concentrations of PMD are being compared to Picaridin 20%).

Sample size calculation outcomes (with Bonferroni correction) when comparing between two PMD midge repellents and a Picaridin positive control.

Assumption of difference in mean CPT between any PMD group with Picaridin group (minutes)	Number of participants required per arm		
	Alpha = 5% Power = 70%	Alpha = 5% Power = 80%	Alpha = 5% Power = 90%
60	21	26	33
80	12	15	19
100	8	10	12
120	6	7	9
180	3	3	4

If only two arms are compared a Bonferroni correction would not be necessary and the numbers of participants needed for the study are slightly smaller.

Sample size calculation outcomes with the assumption of difference in mean CPT between one midge repellent and a Picaridin positive control.

Assumption of difference in mean CPT between PMD and Picaridin group (minutes)	**Number of participants required per arm**		
	Alpha = 5% Power = 70%	**Alpha = 5% Power = 80%**	**Alpha = 5% Power = 90%**
60	17	21	28
80	10	12	16
100	6	8	10
120	5	6	7
180	2	3	4