

Methods and compositions

Technical field

- 5 The present invention relates generally to methods and materials for use in repelling, or avoiding attracting, biting insects.

Background art

- 10 There are numerous publications concerned with the provision of compositions which are said to generally repel or attract (for example to traps) biting insects. These include US 3,668,226; GB 899,289; US 6,444,216; US 6,660,775; WO 03/009688; abstract for JP2002308706; abstract for JP10117657; abstract for JP10139602; US 4,560,551; WO 02/13607; US 6,203,786; abstract for JP11060421; abstract for JP7126110; GB 925,243;
15 abstract for JP20002363006; abstract for JP1019004; WO 02/71840; abstract for JP9030906; US 6,660,288.

- 20 Interestingly, mosquitoes (Culicidae), midges (Ceratopogonidae) and other flies (Diptera) that bite a range of vertebrate hosts to obtain blood, demonstrate preferences between species and within a host species. However there have been relatively few studies which have investigated inter- and intra-species host-preferences in these organisms ^{1-9, 14}.

- 25 Many factors are believed to determine preferences, but for human hosts, studies have demonstrated that differential attraction is determined by volatile olfactory (i.e. volatile chemical) cues that emanate from the skin and associated microorganisms, or breath ^{15, 16}. Specifically, recent evidence suggests that relative attractiveness is caused by differences in olfactory cues provided by individual hosts ¹⁰⁻¹³. However the actual chemical basis for relative attractiveness has remained undefined.

- 30 The provision of novel compositions or uses for controlling the behaviour of insects and inhibiting the activity of biting insects against hosts to which they are attracted would provides a contribution to the art.

Summary of Invention

In one aspect, there is provided the use of a composition comprising geranylacetone as an active ingredient plus a further active ingredient which is 6-methyl-5-hepten-2-one in the preparation of an inhibitory formulation for the treatment of a subject for inhibiting the activity of a haematophagous arthropod against a subject.

In another aspect, there is provided a formulation for treatment of a subject for inhibiting the activity of a haematophagous arthropod against the subject, which formulation is an ointment, solid or solution adapted for topical application, and which comprises:

- (i) a dermatologically acceptable carrier; and
- (ii) a composition comprising geranylacetone as an active ingredient plus a further active ingredient which is 6-methyl-5-hepten-2-one.

In yet another aspect, there is provided an article of manufacture comprising:

- (i) a topical formulation as defined above,
- (ii) aerosol or other spray means for administering the formulation topically.

In yet another aspect, there is provided an article of manufacture comprising:

- (i) a topical formulation as defined above,
- ii) instructions for use of the formulation against the haematophagous arthropod.

In yet another aspect, there is provided an article of manufacture for inhibiting the activity of a haematophagous arthropod against a subject,

which the article is for wearing by or otherwise protecting the subject,

wherein the article comprises a composition which comprises geranylacetone as an active ingredient plus a further active ingredient which is 6-methyl-5-hepten-2-one.

In yet another aspect, there is provided an article as defined above wherein the composition is as defined above.

In yet another aspect, there is provided a method of repelling a haematophagous arthropod from an environment or preventing attraction to an environment,

the method comprising applying to the environment a composition which comprises geranylacetone as an active ingredient plus a further active ingredient which is 6-methyl-5-hepten-2-one.

In yet another aspect, there is provided a dispenser for repelling a haematophagous arthropod from an environment, the dispenser comprising a composition which comprises geranylacetone as an active ingredient plus a further active ingredient which is 6-methyl-5-hepten-2-one.

In yet another aspect, there is provided a method of dispensing an insect repellent into an environment, which method comprises providing a dispenser as defined above into said environment such that the composition is dispensed therefrom.

In yet another aspect, there is provided a use, article or dispenser as defined above wherein an active ingredient is in the form of a precursor.

Disclosure of the invention

5 The present inventors have for the first time demonstrated, counterintuitively, that a repellent, or attractant masking, mechanism is involved in the unattractiveness of individuals to the yellow fever mosquito *Aedes aegypti* and notorious Scottish biting midge, *Culicoides impunctatus*. This is contrary to the assumption in much of the literature that preferences may be dictated by a depleted range of attractants i.e. the view that such 'unattractive' individuals simply did not secrete such attractants.

10 Briefly, the inventors first established differential attractiveness for the yellow fever mosquito, *Ae. aegypti*, within a group of human volunteers and then collected volatile extracts from their bodies using a novel collection technique. Electrophysiological recordings from the antennae of *Ae. aegypti* and *C. impunctatus*, revealed 23 active
15 compounds, which were identified and quantified. Several chemicals were found to be produced in greater amounts by unattractive individuals and these were further investigated as set out in the Examples below.

20 The results showed that some of these compounds caused a significant reduction in flight activity of *Ae. aegypti* when presented together with a human hand in the Y-tube olfactometer. A field trial was conducted against *C. impunctatus* and revealed that some of the active compounds were more effectively repellent, at naturally occurring concentrations, than the commercial biting fly repellent DEET. Compounds described herein may thus be used for protection against such insects.

25 An oral presentation was made at the SOVE (Society of Vector Ecology), 4th International Congress of Vector Ecology, Nugget Hotel/Casino Reno, Nevada, October 2-7, 2005 (Thursday, October 6, 2005; Student Paper Session II - Oral Presentations; SP-16 "Laboratory and field experiments on the effects of synthetic odor blends on the
30 behaviour of the malaria mosquito *Anopheles gambiae*" Qui, T.Yu, yu.tongqiu@wur.nl, Wageningen University, Wageningen, The Netherlands, and R.Smallegange, J.Spitzen, J.Cajo, T.Braak, J.Joop, Var Loon, M.Jawara, P.Milligan, B.Knols, W.Takken). The presentation discussed olfactometer and field tests of a number of compounds believed to occur in human fresh and incubated sweat. Certain compounds, including compounds
35 identified by the present inventors below, were shown to be both attractive and repellent in trials.

A thesis (Qiu, Y.T. (2005) "Sensory and behavioural responses of the malaria mosquito *Anopheles gambiae* to human odours". PhD thesis, Wageningen University, The Netherlands) has also in published in December 2005.

No comparison of 'attractive' and 'unattractive' individuals was made in these disclosures. No trials based on actual application to host skin were conducted.

Generally the invention provides for methods of controlling the behaviour of insects, the method comprising use of a composition of the invention, as described below, which could for example be applied to a subject or environment.

Thus in one aspect the invention provides a method of inhibiting the activity of an insect pest against a subject, the method comprising applying to a subject a composition of the invention, as described below.

The subject to whom the composition is applied will generally be a species which is a normal host target for the insect pest, and would (in the absence of the composition) otherwise be targeted or attract the insect pest. Subjects to be treated with compounds of the present invention could in principle be human or animal subjects (e. g., dogs, cats, horses, cattle). Preferably the subject is a human subject.

The insect pest is preferably a haematophagous insect e.g. of the family Ceratopogonidae (preferably the genus *Culicoides*), or family Culicidae. Preferably the pest is an insect selected from Table 1.

Most preferably it is a mosquito or midge.

Biting midges belong to the genus *Culicoides* of the family Ceratopogonidae; they are the smallest of the bloodsucking insects and are common pests in the UK, NE United States (where they are called punkies, sand flies, and no-see-ums) and many other places around the world.

The composition will be applied in an amount effective to inhibit the activity of an insect pest against the subject, for example by comparison with the same subject without the

composition as determined by standard field methods (such as are used in the Example below; see Ref 18) after any of 1, 5, or 30 minutes.

5 The examples described below show that compositions of the invention can be used to inhibit attraction towards subjects (for example as demonstrated by significant reduction in upwind flight activity towards the subject). Compositions in the field demonstrated repellency based on a World Health Organisation protocol for testing repellents. Thus it is clear that compositions of the invention can be used to negatively affect host-seeking behaviour by insect pests e.g. reduce the attraction to a subject, or increase the repulsion
10 from the subject, and the terms "inhibition of activity" or "repellent" or the like as used herein will be understood by those skilled in the art accordingly.

Compositions of the invention comprise, or consist essentially of, or consist of, geranylacetone as an active ingredient plus preferably a carrier. The geranylacetone will
15 be at a functionally effective amount to inhibit the activity of the insect when in use. Carriers are discussed in more detail hereinafter.

Geranylacetone is alternatively known as 6,10-dimethyl-5,9-undecadien-2-one (cis and trans); 5,9-Undecadien-2-one; 6,10-dimethyl-, trans-Geranylacetone; Nerylacetone;
20 Dihydropseudoionone; 6,10-Dimethyl-undeca-5,9-dien-2-one.

Lwande *et al.* (1999) *Phytochemistry* 50: 401-405 analysed the repellency of the essential oil of the anti-tick pasture shrub *Gynandropsis gynandra* in a tick-climbing repellency bioassay. Constituents of the oil were evaluated and included trans-geranylacetone,
25 nonenal and many others. It was concluded that the blend of all constituents might act as a general purpose arthropod repellent, although no evidence was offered that this would be effective when actually applied to a host.

The inhibition of activity in the field demonstrated herein for geranylacetone containing
30 compositions is surprising since this has previously been discussed in the context of an attractant for clothes moths (see WO 03/009688) and mites (see abstract for JP2002308706; abstract for JP10117657).

In another aspect the compositions of the invention may comprise, or consist essentially of, or consist of, decanal.

The compositions of the invention may comprise, or consist essentially of, or consist of, geranylacetone in combination with one or more other active ingredients defined by the present inventors.

5 In one embodiment the compositions of the invention may include geranylacetone and 6-methyl-5-hepten-2-one. Such compositions of the invention may also include one or more further compounds selected from the list consisting of: octanal; decanal; nonanal.

10 6-Methyl-5-hepten-2-one is alternatively known as 6-Methylhept-5-en-2-one; Sulcatone; 5-Hepten-2-one, 6-methyl-; 6-Methyl-5-heptene-2-one; 2-Methyl-2-hepten-6-one; -Methyl hept-5-en-2-one; Methylheptenone; 2-Methyl-6-oxo-2-heptene; 2-Oxo-6-methylhept-5-ene.

15 In one embodiment the compositions of the invention may include geranylacetone and octanal. Such compositions of the invention may also include one or more further compounds selected from the list consisting of: 6-methyl-5-hepten-2-one; decanal; nonanal.

20 All of the above compounds have shown inhibitory activity in the Examples hereinafter.

Optionally the compositions of the invention also include one or more further compounds selected from the list consisting of: benzaldehyde; naphthalene; acetophenone; (E)-2-octenal).

25 These compounds have been shown to be present in higher amounts in the skin of 'unattractive' individuals. They may optionally be added to the compositions of the present invention.

30 The compositions may optionally exclude ingredients such as one or more of: limonene, m-Cymene, 1-alpha-terpineol, beta-cyclocitral, nerol, carvacrol, beta-ionone, nerolidol, and in particular Methyl isothiocyanate. The compositions may exclude ingredients such as one or more of: heptanoic acid, tetradecanoic acid, 3-methyl-1-butanol, 4-ethylpentone.

35 Thus, in addition to optional further active ingredients, additives, and a carrier as discussed hereinafter, preferred compositions of the present invention comprise

geranylacetone in the composition in a concentration of at least about 0.0001% by weight, but may be up to 10, 20, 50, or 99.9 % by weight of the total composition.

The carrier may be from 0.1% to 99.9999% by weight of the total composition.

Preferably the compositions of the invention comprise:

Geranylacetone:	0.0001 - 10%
more preferably:	0.001 - 10%

and optionally one or more further additive compounds selected from the list consisting of:

6-Methyl-5-hepten-2-one:	0.00001 - 10%
Octanal	0.00001 - 1%
Nonanal	0.1 - 10%
Decanal	0.0001 - 10%

plus optional further active ingredients, additives, and a carrier as discussed hereinafter in order to make 100%. '%' in this context means g/100g (solid compositions, total weight) or g/100 ml (liquids or ointments, total volume).

Based on the examples set out below, such concentrations of ingredients may be employed for inhibition of flight towards a target.

Based on experiments showing orientation away from the source, compositions of the present invention may in certain embodiments comprise:

Geranylacetone:	0.0001 - 10%
and optionally :	
Decanal	0.0001 - 0.1 %

Compositions of the present invention may comprise:

Geranylacetone:	0.001% to 1%
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and optionally one or more further compounds selected from the list consisting of:

6-Methyl-5-hepten-2-one: 0.001% and 1%

Decanal: 1%

Octanal: 0.01%

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Examples of such compositions have been shown to be effective against biting midges in field trials.

10 A preferred mixture for use in the compositions of the present invention will comprise Geranylacetone plus at least one, two, three, four, or five of the preferred additive compounds described above.

A preferred mixture for use in the compositions of the present invention is as follows:

15 Geranylacetone: 0.00001 - 10%, more preferably 0.001 - 10%, more preferably 0.01 - 10%, more preferably 0.1 - 5%, most preferably about 1%, and.

6-Methyl-5-hepten-2-one: 0.1 - 10%, more preferably 0.1 - 5%, most preferably about 1%.

20 Another preferred mixture for use in the compositions of the present invention is as follows:

Geranylacetone: 0.00001 - 10%, more preferably 0.001 - 10%, more preferably 0.01 - 10%, more preferably 0.1 - 5%, most preferably about 1%.

25 6-Methyl-5-hepten-2-one: 0.1 - 10%, more preferably 0.5 - 5%, most preferably about 1%.

Octanal: 0.001 - 0.1%, more preferably 0.005 - 0.05%, most preferably about 0.01%.

30 Based on analysis of 'attractive' and 'unattractive' individuals, for compositions comprising 6-Methyl-5-hepten-2-one and Geranylacetone it is preferred that these are in a weight ratio of at least or equal to about 0.5:1, more preferably at least or equal to about 1:1; 1.5:1; 2:1; 20:1; 200:1 or more.

Other preferred mixtures are discussed in the Examples hereinafter.

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Compositions of the present invention may be formulated as ointments or solutions which contain the active ingredients disclosed herein.

5 The invention further provides use of geranylacetone, preferably in the form of any composition of the invention (as described herein), in the preparation of an inhibitory formulation for treatment of a subject for inhibiting the activity of an insect pest against the subject.

10 Preferably the composition is for topical application to the subject. For topical application, the formulation may take the form of a spray formulation or a cream or lotion formulation.

15 Thus the compositions of the present invention may be employed with such solid and/or liquid dispersible carrier vehicles as described herein or as otherwise known in the art, and/or with other known compatible active agents, if desired, in the form of particular dosage preparations for specific application made therefrom, such as solutions, emulsions, suspensions, powders, pastes, and granules as described herein or as otherwise known in the art which are thus ready for use. The formulation may include antioxidants and UV absorbers.

20 Liquid formulations may be aqueous-based or non-aqueous(i. e., organic solvents such as ethanol as used in the Examples below), or combinations thereof, and may be employed as foams, gels, suspensions, emulsions, microemulsions or emulsifiable concentrates or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants or polymers.

25 US 6,444,216 discusses the use of volatile silicon fluids to impart mildness and water repellency to insect repellent compositions. As noted therein volatile silicon fluids are available commercially. For example it is reported that Dow Corning uses commercial designations of 244, 245, 246, 344 and 345, which are mixtures of
30 polydimethylcyclsiloxanes (cyclomethicones) and are composed of tetramers (e.g. cyclotetrasiloxane, octamethylcyclotetrasiloxane), pentamers (e.g. cyclopentasiloxane, decamethylcyclopentasiloxane), and hexamers (e.g. cyclohexasiloxane, dodecamethylcyclohexasiloxane). Mixtures of the various silicone fluids, either with other silicone fluids or non-silicon containing substances, are used in a variety of cosmetic
35 preparations to impart special properties, to include water repellency and skin protection.

The formulations may be a solid mixture which releases a vapour having the same composition of active ingredient or ingredients as the solid mixture. Agents to promote sublimation of active ingredients to achieve this are described in GB 925,243.

5 Thus the present invention also provides a topical formulation for inhibiting the activity of an insect pest against a subject, which formulation consists essentially of a composition a composition of the present invention as described above.

10 The present invention also provides a topical formulation for inhibiting the activity of an insect pest against a subject, which formulation comprises:

- (i) a dermatologically acceptable carrier; and
- (ii) a composition of the present invention as described above,
- (iii) optionally one or more active ingredients,

15 The present invention also provides an article of manufacture comprising:

- (i) a topical formulation for inhibiting the activity of an insect pest against a subject as described above,
- (ii) aerosol or other spay means for administering the formulation topically.

20 Insect repellent compositions are available commercially in aerosol form and aerosol technologies are well known in the art. Consumers find aerosol compositions easy to administer to both the skin and to clothing. Further, aerosol compositions help consumers avoid the inconvenience of having to apply and spread the insect repellent with the hands. US 6,969,521 relates to cosmetically-acceptable aerosol vehicles to disperse
25 and deliver the insect repellent active, the vehicle having a volatile organic compound component.

The present invention also provides an article of manufacture comprising:

- (i) a topical formulation for inhibiting the activity of an insect pest against a subject as
30 described above,
- (ii) instructions for use of the formulation against the insect pest.

35 When using the formulations of the present invention, subjects may be directly or indirectly treated, such as by applying the formulation to the skin of the subject, or by applying the formulation to an article worn by or otherwise protecting the subject. For example garments, belts, collars, or other articles worn by the subject from whom insects

are to be repelled. Preferred examples may include a slow-release badge worn on clothing; arm, wrist or ankle bands; accessories including pendants, necklaces, bracelets.

5 The formulation may be applied to netting or screening that protects a subject, particularly a sleeping subject.

In another alternative, the compositions of the invention may be formulated in paints with a conventional microencapsulation technology.

10 The repellent compounds are suitably applied by any method known in the art including, for example, spraying, pouring, dipping, in the form of concentrated liquids, solutions, suspensions, sprays, powders, pellets, briquettes, bricks and the like, formulated to deliver a repellent effective concentration of the repellent compound. It will be recognized that the concentration, applied amount and frequency of application will vary with the
15 subject and locus of application, e. g., to the skin or hair of a human.

The invention provides for applying the insect repellent formulation to achieve a topical dose of any given active ingredient of between about 0.0025ug/cm² to 100 ug/cm², more preferably 0.25ug/cm² to 25 ug/cm². One preferred final concentration is around 10
20 ug/cm² Based on the application of around 1 ml to a forearm of approximately 400 cm², this can be achieved by a concentration of around:

0.0001% = 0.0025ug/cm²

0.01% = 0.25ug/cm²

25 1% = 25ug/cm²

4% = 100ug/cm²

As noted above, when using the formulations of the present invention, subjects may not be directly treated. Thus the compositions may be used or applied to an environment
30 (e.g. a pre-defined region, which may or may not be contained) from which it is desired to repel pests (or mask the attractiveness of the environment to pests). Thus as noted above the formulation may be applied to netting or screening that protects a subject. Alternatively it may be provided direct to an environment e.g. by spraying or by use of compositions of apparatus adapted to deliver a repellent effective concentration of the
35 repellent compound.

Thus it will be understood the invention provides a method of repelling an insect pest from an environment or preventing attraction to an environment (e.g. by masking the attractiveness if a subject therein), the method comprising applying to the environment a composition of the present invention e.g. comprising geranylacetone as an active ingredient plus a carrier plus optionally a further active ingredient selected from the list consisting of: 6-methyl-5-hepten-2-one, octanal; decanal; nonanal. The compositions may be any of those discussed above or below.

Application (or dispensing) to an environment may be achieved by conventional means which include (by way of example only): candles, coils, slow release dispensers, (e.g. electric and non-electric vaporisers), electric and non-electric oil burners, electric 'plug-in' diffusers. Numerous such dispensers for volatile substances are very well known to those skilled in the art e.g. for dispensing incense, perfume, medicaments, insecticides, insect repellents, disinfectants, or fumigants. In such dispensers the volatile substance may be formulated as a gel for slow release e.g. with a gelling agent selected from the group consisting of dibenzylidene sorbital acetal, gellan gum and polyacrylic acid polymers. Any such known dispenser or formulation may be applied analogously in the present invention.

Any such dispenser, comprising (and adapted to dispense) a composition of the present invention (optionally in the form of a gel) provides a further aspect of the present invention, as does a method of dispensing a composition of the present invention comprising use of any such dispenser.

One preferred dispenser is an electrostatic chemical dispenser such as is available from Aerstream Technology Limited (Wallingford, UK). In such a system droplets are electrostatically charged such that they may pervade an environment longer than aerosols. One such system is described in International Patent Application WO03/000431.

Thus an electrostatic chemical dispenser according to the present invention may comprise

- a) a conduit that contains, in use, liquid to be atomised, which liquid comprises, consists or consists essentially of a composition of the present invention;
- b) a spray electrode; and,
- c) a discharge electrode.

Briefly, in such a system, each electrode is adjacent to a dielectric, wherein the electrodes are connected in an electrical circuit to enable a potential difference to be applied between the spray electrode and the discharge electrode to atomise the liquid and to generate charge carriers of a first polarity in the proximity of the spray electrode and ions of a second polarity in the proximity of the discharge electrode, and wherein some of the first polarity charge carriers deposit on the dielectric adjacent to the spray electrode and some of the second polarity ions deposit on the dielectric adjacent to the discharge electrode so that the atomised liquid is repelled from the dielectric adjacent to the spray electrode and electrically discharged by second polarity ions that are repelled by the dielectric adjacent to the discharge electrode.

It will be understood that any of the active ingredients of the compositions of the present invention may be provided in precursor form. A precursor molecule in this context is one which, during or following application to the skin, is converted to the relevant chemical constituent. Precursors of the compounds specified herein may be readily provided by those skilled in the art based on well established principles - for example compounds containing an olefinic double bond which on oxidation yield the ketone or aldehyde group for these compounds could be utilised. For the ketones, precursors could be squalene or a lower molecular isoprenoid with the appropriate structural requirements and for the aldehydes an unsaturated carboxylic acid with the double bond at $\omega 8$, $\omega 9$, $\omega 10$ could be used.

Precursors molecules may be desired to manipulate properties such as stability, skin retention (i.e. rate of release) and so on. So called "dermal anchoring" molecules are discussed for example in US 3,668,226.

The disclosure herein applies *mutatis mutandis* to the use of such precursor molecules as it does to geranylacetone; 6-methyl-5-hepten-2-one; octanal; nonanal; decanal.

The invention will now be further described with reference to the following non-limiting Figures and Examples. Other embodiments of the invention will occur to those skilled in the art in the light of these.

The disclosure of all references cited herein, inasmuch as it may be used by those skilled in the art to carry out the invention, is hereby specifically incorporated herein by cross-reference.

5 Figures

Figure 1. Behavioural responses *Aedes aegypti* host-seeking female mosquitoes to the hands of volunteers. a) Flight activity - the proportion of mosquitoes that were recorded upwind in the Y-tube; b) Relative attraction - the proportion of mosquitoes that were
10 recorded in the 'test' arm of the Y-tube. Data are presented as differences between the response to the volunteer and the response to the standard stimulus (hand of volunteer Y01). Back transformed means $\pm$ s.e.d. are shown. Asterisks indicate statistically significant differences from the standard (Y01) (GLM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns not significant; $n = 10-12$).

Figure 2. Behavioural responses *Aedes aegypti* host-seeking female mosquitoes to extracts (+CO₂) collected from seven volunteers and the standard volunteer (Y01). a) Flight activity - the proportion of mosquitoes that were recorded upwind in the Y-tube; b) Relative attraction - the proportion of mosquitoes that were recorded in the 'test' arm of
20 the Y-tube. Back transformed means $\pm$ confidence intervals (95 %) are shown. Asterisks indicate statistically significant differences from the control (GLM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns not significant; $n = 10-12$).

Figure 3. Typical coupled GC-EAG traces a). *Aedes aegypti* female mosquito response
25 to human (Y01, attractive) air entrainment extract and *C. impunctatus* midge responses to human (Y05, unattractive) air entrainment extract. Top trace corresponds to the FID detector on the GC. Bottom trace corresponds to the antennal response of the insect preparation. A. benzaldehyde B. 6-methyl-5-hepten-2-one, C. octanal, D. unknown 7, E. nonanal & linalool, F. menthol & naphthalene, G. decanal, H. geranylacetone.

Figure 4. (a) Absolute total (ng), and
30 (b) mean amounts (%) of EAG active compounds collected from whole body air entrainments of volunteers within the attractive group ($n = 6$) and unattractive group ($n = 4$). Significant differences between the two means are denoted by: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Compound numbers: 1. hexanal, 2. benzaldehyde, 3. 6-methyl-5-hepten-2-one, 4. octanal, 5. 1,4-dichlorobenzene, 6. 2-ethylhexanol, 7. limonene, 8. unknown 7, 9.

dihydromyrcenol, 10. nonanal, 11. linalool, 12. undecane, 13. (*E*)-2-nonenal, 14. octanoic acid, 15. menthol, 16. naphthalene, 17. decanal, 18. indole, 19. dodecanal, 20. geranylacetone, 21. pentadecane, 22. hexadecane, 23. α -isomethylionone, 24. heptadecene. Significant differences between the two groups are denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

(c) Plot of canonical-variate scores based on the amounts of all GC-EAG active chemicals within air entrainment extracts for all volunteers. Black crosses = Group 1 (attractive individuals); Red crosses = Group 2 (unattractive individuals). Circles represent 95 % confidence intervals.

Figure 5. Mean proportion of *Aedes aegypti* female mosquitoes showing flight activity in the Y-tube in response to the standard (hand of Y01 in one side of the Y-tube and filter paper control in the other) and seven doses of a) 6-methyl-5-hepten-2-one, b) octanal, c) nonanal, d) decanal and e) geranylacetone chemical treatments (hand of Y01 + chemical in one side of the Y-tube and filter paper solvent control in the other). ($-1 = 1 \times 10^{-1} \text{ g } \mu\text{L}^{-1}$; $-2 = 1 \times 10^{-2} \text{ g } \mu\text{L}^{-1}$, etc). Back transformed means $\pm$ 95 % confidence intervals (calculated for a binomial distribution) from the logistic scale are shown.

Figure 6. Repellency of EAG-active human-derived compounds applied to the forearms of volunteers and tested on *Culicoides impuctatus* midges in the field. a) over 1 minute and b) over 5 minutes.

Examples

Example 1- defining attractive and unattractive volunteers

A convenient bioassay was established following work by Geier *et al.* (1999)¹⁷ using a Y-tube olfactometer in which mosquitoes, *Ae. aegypti*, could "choose" between volatile skin emanations and air alone, the "choice" being expressed as flight activity or relative attraction. Initially, the source of skin emanations was from individual hands of a series of human volunteers. Volunteers were analysed in this way until a range of attractive and unattractive examples were obtained (Fig. 1). Five volunteers (X04, X07, X09, X08 and X05) induced greater upwind flight activity and relative attraction than the standard volunteer (Y01). Four volunteers (X06, Y04, Y05 and Y07) induced significantly less upwind flight activity and relative attraction than the standard (Y01).

Example 2 – obtaining volatile chemicals from volunteers

A novel system was devised for obtaining volatile chemicals from whole bodies. This involved placing each volunteer (and the standard (Y01)) from the attractive and unattractive groups in an aluminiumised plastic bag (Lifesystems™), with the head outside, under positive pressure from clean air and withdrawing air that had passed over the body, to be entrained through a small column of absorbent polymer. This provided samples, the same batch of which could be used for multiple behavioural, electrophysiological and chemical analysis.

Example 3 – confirmation of validity of volatile chemicals from volunteers

The volatiles were extracted from the polymer and assayed again using the Y-tube olfactometer against an air control and standard volunteer (Y01), all with added CO₂ (Fig. 2). For the first time, volatile extracts of human volunteers were shown to exhibit similar attractiveness or unattractiveness to the direct human skin emanations, with extracts from the standard (Y01), X04, X07 and X09 being significantly attractive, and those from X06, Y04, Y05 and Y07 as unattractive as the air control.

Example 4 – comparison and analysis of volatile extracts

The volatile extracts of the volunteers used in Figure 2 were then by high resolution GC directly coupled whole antennal electrophysiological preparations, from female *Ae. aegypti*, (6-12 days old) to produce GC-electronantennograms (GC-EAG), typically as shown in Figure 3. Thus, components which were physiologically active, and therefore likely to be responsible for the differences in attractiveness of the human volunteers, were determined.

Compounds giving the peaks associated with electrophysiological activity by GC-EAG were tentatively identified by GC-MS under similar conditions and the identity confirmed by peak enhancement on GC using two columns of different polarity by coinjection with authentic compounds.

Identifications are listed in Table 2 and absolute amounts, determined by a multiple point external standard method, given. Statistically significant differences between the attractive (Group 1) and unattractive (Group 2) were observed for:

- benzaldehyde,
- 6-methyl-5-hepten-2-one,
- octanal,
- 5 • nonanal,
- decanal,
- naphthalene
- geranylacetone

10 with the levels being higher, in Group 2.

Additionally the relative amounts (in respect of total chemical composition; see Fig. 4) of 6-methyl-5-hepten-2-one, octanal and decanal were higher.

15 A number of electrophysiologically active compounds from the 32 active peaks have not been conclusively identified, but only one was believed to be associated with unattractiveness.

- unknown 7 (believed to be acetophenone or (E)-2-octenal).

20 Although a number of electrophysiologically active compounds were chiral the isomeric composition was not determined as these compounds were not implicated as factors contributing to human unattractiveness. Care was made to remove contamination of volunteers, for example with industrial or cosmetic chemicals (see Methods).

25 Nevertheless compounds with electrophysiological activity were shown even if, as with dichlorobenzene and naphthalene, these could have arisen from synthetic sources.

30 Confirming the grouping of the volunteers, multivariate analysis revealed that the attractive (Group 1) and unattractive (Group 2) groups differed significantly in terms of the variability in the amounts of the seven specified EAG-active chemicals between the two groups (Fig. 4; Table 1).

Tables 3a and 3b show ratio of these compounds from "attractive" volunteers and "unattractive" volunteers.

Example 5 – testing of candidate compounds

Based on the results above, five chemicals were chosen as those most likely to be naturally involved in human unattractiveness and were subjected to further analysis:

- (i) 6-methyl-5-hepten-2-one,
- (ii) octanal,
- (iii) nonanal,
- (iv) decanal,
- (v) geranylacetone

These compounds (from Sigma Aldrich) were tested individually at different concentrations in the olfactometer against *Ae. aegypti*. The upwind flight activity, relative attraction and probing activity (feeding) of the mosquitoes to the hand of an attractive volunteer with and without the chemicals was recorded. All chemicals caused a significant reduction in flight activity and geranylacetone caused a reduction in relative attraction (Fig. 5; data for relative attraction not shown). Probing activity (feeding) was also significantly reduced by 6-methyl-5-hepten-2-one and geranylacetone (data not shown).

Thus, it is clear that each of these five demonstrated the ability to interfere significantly with the attractiveness of skin emanations from the hand of an attractive standard volunteer, i.e. Y01, at naturally occurring levels. Only nonanal allowed significantly greater flight activity than the untreated hand but at only one dose.

Only octanal has been described previously as a repellent for *Ae. aegypti* (H. D. Douglas *et al.* "Chemical Odorant of Colonial Seabird Repels Mosquitoes" *J. Med. Entomol.* 42(4): 647-651 (2005)), and the compounds newly identified here as repellents could be used in defence against these other biting flies and possibly other haematophagous arthropods.

The chemical factors conferring low human attractiveness to mosquitoes and midges determined here could be still more effective given a more persistent formulation.

Interestingly, the variation between DEET and a more recent repellent, picaridine (Bayrepel ®), shows variability of repellency with human subjects¹⁹. This may arise in part from the type of individual variation described herein.

Example 6 – further analysis of volatile extracts

The extracts of volunteer human volatiles were also analysed by GC-EAG on *C. impunctatus*, which it is understood is the first time such an investigation had been done with a Ceratopogonid. A typical trace is given in Figure 3 and the same range of compounds was responded to, but particularly those implicated as giving negative responses in samples from the unattractive volunteers.

Example 7 – field trials of compounds

A field trial was made in the Ormsary estate, Argyllshire, during the summer 2005 in a region with an extremely high natural population of *C. impunctatus*. Active compounds at reducing normal human attractiveness to *Ae. aegypti* in the Y-tube olfactometer (6-methyl-5-hepten-2-one; geranylacetone) plus six mixtures, were tested for repellency (Table 4) using a WHO protocol¹⁸. Compounds were applied to (nylon) stockings and worn on the forearms of volunteers and were tested at three concentrations in ethanol (1%, 0.01% and 0.0001%). All compounds and mixtures were compared against the WHO approved repellent diethyltoluamide (DEET) (1% and 40%).

Results (Fig. 6) show that DEET at 40% was not more repellent than DEET at 1%. Therefore, statistical comparisons were subsequently made between the compounds or mixtures and DEET at 1%.

Individual compounds

Geranylacetone was effective at all concentrations tested and with a typical dose response of increasing efficacy with increasing concentration.

For 6-methyl-5-hepten-2-one at the 1 min time, at 0.001% is significantly repellent; 0.01% is not repellent but 1% is strongly repellent, indeed more effective than DEET, but was repellent only at 1% over 5 min.

Over 1 min, 1% geranylacetone, 1% 6-methyl-5-hepten-2-one, Mix 5 and Mix 6 were significantly greater than DEET at 1%, and for the full 5 min testing period.

1% geranylacetone, Mix 6 and Mix 5 showed higher % repellency than DEET; these differences were not significant.

Example 8 – additional field trials

Methods

Human-derived compounds (6-methyl-5-hepten-2-one and geranylacetone) were tested for repellency against *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes in laboratory-based repellency tests using an established protocol (Omolo et al., 2004). The mosquitoes were laboratory-reared using standard conditions at ICIPE, Duguru. 5-7 day old female mosquitoes that had been starved for 18h but previously fed on 6% glucose solution were used. Eighteen cages (50x50x50cm) were used with 25 female mosquitoes in each. Six human volunteers with no or little allergic reaction to bites were selected. Test solutions (0.5ml in acetone) were applied to a volunteer's forearms and the hand was covered with a glove. Acetone alone (0.5ml) served as a control on the other arm. The control arm was inserted into a cage and the number of landings were recorded over 3 min. The treated arm was then inserted as above. Repellency data was expressed as protective efficacy (PE) and was calculated by using the formula $PE = (\% \text{ control mean} - \% \text{ test mean} / \% \text{ control mean})$. The data were transformed and subjected to an ANOVA.

Each compound was tested at concentrations ranging from 0.0001% to 10%.

Three mixtures were also tested at the same concentrations and comprised the following components:

Mixture 1: 1:1:1:1:1 of 6-methyl-5-hepten-2-one, octanal, nonanal, decanal and geranylacetone

Mixture 2: 1:3:1:0.5:0.5 of 6-methyl-5-hepten-2-one, octanal, nonanal, decanal and geranylacetone

Mixture 3: 1:1 of 6-methyl-5-hepten-2-one and geranylacetone.

Mixture 1 and mixture 3 were then incorporated into a formulation which consisted of emulsifying wax NF, Petroleum jelly and liquid paraffin in a 1.2:2.8:1 ratio. These formulated mixtures were then tested using the above methodology and were repeated after 2, 4, 6 and 8 hours to give PE over time.

Results

The human-derived compounds tested against *Anopheles gambiae* s.s. gave dose-dependent repellency, with the maximum repellency observed for compounds at 10%. 100% repellency was recorded only for geranylacetone at 10%.

DEET gave better repellency than the single compounds giving 100% repellency at 1% and 10% (Tables 5-7). However, the greatest repellency against this species was given by mixture 3, which achieved 100% repellency at concentrations of 1% and 10%.

At lower concentrations than these, good repellency was also observed and at 0.1 % concentration 87% repellency was recorded in comparison with DEET which gave only 83% repellency. At a lower concentration (0.01%) mixture 3 gave 80% repellency compared with DEET which gave only 20% repellency at this concentration (Tables 8-10). All formulated mixtures gave 100% repellency at the start of the experiments (i.e. time zero). Around 90% repellency was achieved after 2 hours with mixture 1 and this decreased over time to 12% after 8 hours (Tables 11-13). Greater repellency was observed for mixture 3 with 98% repellency recorded after 2 hours. Repellency then decreased to 64% after 4 hours and to 35% after 8 hours. DEET maintained 100% effectiveness up to 6 hours and 94% after 8 hours.

The human-derived compounds tested against *Culex quinquefasciatus* gave dose-dependent repellency, with the highest repellency for each compound observed at 10%. Geranylacetone and DEET gave 100% repellency but only at a concentration of 10% (Tables 14-16).

Mixture 1 and 3 gave 100% repellency at a concentration of 10%. At lower concentrations, mixture 1 gave greater repellency than mixture 3 with 84% and 77% repellency achieved respectively at a concentration of 1%. Both formulated mixtures gave 100% repellency at the start of the experiment, and this decreased slightly to 89% for mixture 1 and to 99% for mixture 3 after 2 hours (Tables 17 and 18).

After 8 hours, the repellency given by mixture 1 had decreased to 20%. However, for mixture 3, repellency was maintained at a greater level, decreasing only to 80% after 4 hours, 60% after 6 hours and 45% after 8 hours. DEET gave 100% repellency up until 6 hours and this decreased to 93% after 8 hours (Tables 19-21).

Conclusions

The most effective treatment against *Anopheles gambiae* over 3 minutes was mixture 3 and this was better than DEET at equivalent concentrations. For *Culex quinquefasciatus*, the most effective treatment was decanal alone at 1%, although this was not better than DEET. The best treatment comprising human-derived compounds was mixture 3 for both species.

Table 1. Insect and Tick targets.

INSECT	DISEASES VECTORED	COUNTRY	IRRITATION TO HOST
<u>Mosquitoes</u>			
<i>Culex pipiens</i> complex	West Nile Virus Japanese Encephalitis Filariasis	USA Asia South America, Africa	Yes
<i>Aedes aegypti</i>	Dengue fever Yellow fever	South America and Africa	Yes
<i>Anopheles</i> <i>stephensi</i>	Malaria	Asia	Yes
<i>Anopheles</i> <i>gambiae</i> complex	Malaria	Africa	Yes
<u>Midges</u>			
<i>Culicoides</i> <i>impunctatus</i>	None	Scotland, UK	Yes
<i>Culicoides</i> <i>molestus</i>	None	Australia	Yes
<u>Ticks</u>			
<i>Ixodid</i> species (<i>Borrelia</i>)	Borreliosis and Lyme disease	EU and USA	No
<u>Sandflies</u>			
<i>Lutzomyia</i> species	Leishmaniasis (visceral, mucosal, cuticular)	EU, Portugal South America	Yes Yes
<i>Phlebotomine</i> species	Leishmaniasis	Africa	Yes
<u>Midges</u>			

Other	Bluetongue	EU and Africa	Yes
<i>Culicoides</i> midges	African Horse Sickness		
<u>Ticks</u>			
<i>Ixodid</i> ticks	Tick-borne encephalitis	North America	No
	Tick-borne fever	Africa	

Table 2. Names and retention indices for 24 EAG-active chemicals with loadings from CVA.

Compound no	Retention Index	Compound name	Latent vectors (loadings)
1	776	Hexanal	0.00044
2	932	Benzaldehyde	0.00133
3	965	6-Methyl-5-hepten-2-one	-0.00427
4	980	Octanal	0.00062
5	988	1,4-Dichlorobenzene	0.00126
6	1010	2-Ethylhexanol	0.00117
7	1022	Limonene	-0.0005
8	1030	Unknown 7	0.00016
9	1057	Dihydromyrcenol	-0.00082
10	1080	Nonanal	-0.00047
11	1083	Linalool	0.00047
12	1100	Undecane	0.0001
13	1135	(E)-2-nonenal	0.00011
14	1151	Octanoic acid	0.00056
15	1160	Menthol	0.00097
16	1160	Naphthalene	-0.00102
17	1184	Decanal	-0.00245
18	1256	Indole	-0.00233
19	1384	Dodecanal	-0.00122
20	1430	Geranylacetone	0.00042
21	1480	α -isomethylionone	0.00037
22	1500	Pentadecane	-0.00067
23	1600	Hexadecane	-0.00024
24	1681	Heptadecene	0.00115

**Table 3a. Ratios of compounds based on mean amounts of chemicals from
"attractive" volunteers and "unattractive" volunteers.**

Compound	Attractive	Unattractive
Benzaldehyde	1	1
6-MHO	2.020595	1.55218
Octanal	0.541551	0.529534
Nonanal	6.510643	2.906716
Naphthalene	0.875217	0.979441
Decanal	1.821094	1.468768
Geranylacetone	5.130115	1.108576

Table 3b. Ratios of 6-methyl-5-hepten-2-one to geranylacetone

The table shows the ratio in one unattractive volunteer and the ratios for the mean absolute amounts of the attractive and unattractive group of volunteers.

	Standard unattractive person		Attractive group		Unattractive group	
	Absolute amount	Ratio	Mean absolute amount	Ratio	Mean absolute amount	R
6-methyl-5- hepten-2-one	350.0	1.8	49.73	0.4	320.08	
Geranylacetone	200	1	126.26	1	228.6	

Table 4. Mixtures of EAG-active human-derived compounds tested on *C. impunctatus* in the field.

Mixture 1	1% 6-methyl-hepten-2-one, 0.01% octanal (in ethanol)
Mixture 2	1% 6-methyl-hepten-2-one, 0.01% octanal, 0.02% geranylacetone (in ethanol)
Mixture 3	1% 6-methyl-hepten-2-one, 1% geranylacetone, 0.01% octanal (in ethanol)
Mixture 4	1% 6-methyl-hepten-2-one, 0.0001% geranylacetone, 0.01% octanal (in ethanol)
Mixture 5	1% 6-methyl-hepten-2-one, 1% geranylacetone (in ethanol)
Mixture 6	1% geranylacetone, 0.01% octanal (in ethanol)

Table 5. Dose- response repellency of geranylacetone decanal tested against *An. gambiae*

% Conc.	0.001	0.01	0.1	1	10
Mean % P.E±S.E	7.25±9.5	11.17±12.9	30.13±11	76.97±5.9	100±0

Table 6. Dose- response repellency of 6-methyl-5-hepten-2-one tested against *An. gambiae*

% Conc.	0.0001	0.001	0.01	0.1	1	10
Mean % P.E±S.E	0.85±3.9	1.57±11.2	19.2±5.5	34.07±6.6	35.73±8.7	74.75±6.1

Table 7. Dose- response repellency of DEET tested against *An. gambiae*

% Conc.	0.001	0.01	0.1	1	10
Mean % P.E±S.E	6.23±1.7	20.65±6.4	83.78±3.9	100±0	100±0

Table 8. Dose- response repellency of Mixture 1: 1:1:1:1:1 of 6MHO:Octanal:Nonanal:Decanal:Geranylacetone tested against *An. gambiae*

% Conc.	0.001	0.01	0.1	1	10
Mean % P.E ± SE	9.2±1.8	16.93±3.5	44.58±1.1	94.1±1.1	100±0

Table 9. Dose- response repellency of Mixture 2: 1:3:1:0.5:0.5 of 6MHO:Octanal:Nonanal:Decanal:Geranylacetone tested against *An. gambiae*

% Conc.	0.001	0.01	0.1	1	10
Mean % P.E ± SE	3.47±6.4	16.50±5.1	26.93±3.7	59.48±3.1	100.00±0

Table 10. Dose- response repellency of Mixture 3: 1:1 of 6MHO:Geranylacetone tested against *An. gambiae*

% Conc.	0.0001	0.001	0.01	0.1	1	10
Mean % P.E ± SE	9.4±2.3	24.38±5.1	79.42±3.7	87.08±3.1	100±0	100±0

Table 11. Dose- response repellency of formulated Mixture 1: 1:1:1:1:1 of 6MHO:Octanal:Nonanal:Decanal:Geranylacetone (10% concentration) tested against *An. gambiae* over 8 hours.

Duration (hrs)	0H	2H	4H	6H	8H
Mean % P.E±S.E	100±0	89.41±3.67	54.87±4.49	32.11±5.59	12.50±2.1

**Table 12. Dose- response repellency of formulated Mixture 3: 1:1 of 6MHO:
Geranylacetone (10% concentration) tested against *An. gambiae* over 8 hours.**

Duration (hrs)	0H	2H	4H	6H	8H
Mean % P.E±S.E	100±0	98.48±1.51	64.26±6.15	38.82±4.31	30.83±6.96

**Table 13. Dose- response repellency of formulated DEET (10% concentration)
tested against *An. gambiae* over 8 hours.**

Duration (hrs)	0H	2H	4H	6H	8H
Mean % P.E±S.E	100±0	100±0	100±0	100±0	94.35±1.02

Table 14. Dose- response repellency of geranylacetone tested against *Culex quinquefasciatus*

% Conc.	0.0001	0.001	0.01	0.1	1	10
Mean % P.E±S.E	4.8±2.4	10±1.6	23.7±4.5	33.2±4.5	84.1±4.2	100±0

**Table 15. Dose- response repellency of 6-methyl-5-hetpen-2-one tested against
*Culex quinquefasciatus***

% Conc.	0.0001	0.001	0.01	0.1	1	10
Mean % P.E±S.E	8.0±1.8	12.7±2.1	21.8±4.9	30.1±4.1	49.7±2.9	100.0±0

Table 16. Dose- response repellency of DEET tested against *Culex quinquefasciatus*

% Conc.	0.001	0.01	0.1	1	10
Mean % P.E±S.E	7.6±1.8	12.0±1.7	76.9±5.4	98.2±1.1	100±0

Table 17. Dose- response repellency of Mixture 1: 1:1:1:1:1 of 6MHO:Octanal:Nonanal:Decanal:Geranylacetone tested against *Culex quinquefasciatus*

Conc.	0.001	0.01	0.1	1	10
Mean % P.E±S.E	9.61±3.53	20.82±4.86	33.46±3.76	84.06±6.94	100±0

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Table 18. Dose- response repellency of Mixture 3: 1:1 of 6MHO: Geranylacetone (at 10% concentration) tested against *Culex quinquefasciatus*

Conc.	0.001	0.01	0.1	1	10
Mean % P.E±S.E	11.27±1.86	20.83±2.26	55.64±5.81	77.00±4.53	100±0

Table 19. Dose- response repellency of formulated Mixture 1: 1:1:1:1:1 of 6MHO:Octanal:Nonanal:Decanal:Geranylacetone (at 10% concentration) tested against *Culex quinquefasciatus* over 8 hours.

Duration (hrs)	0H	2H	4H	6H	8H
Mean % P.E±S.E	100±0	89.03±4.2	56.17±4.37	28.46±5.15	19.83±3.16

Table 20. Dose- response repellency of formulated Mixture 3: 1:1 of 6MHO: Geranylacetone (at 10% concentration) tested against *Culex quinquefasciatus* over 8 hours.

Duration (hrs)	0H	2H	4H	6H	8H
Mean % P.E±S.E	100±0	98.57±1.43	79.97±1.7	60.91±3.1	45.79±4.13

Table 21. Dose- response repellency of formulated DEET (at 10% concentration) tested against *Culex quinquefasciatus* over 8 hours.

Duration (hrs)	0H	2H	4H	6H	8H
Mean % P.E±S.E	100±0	100±0	100±0	100±0	93.33±4.08

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Claims

1. Use of a composition comprising geranylacetone as an active ingredient plus a further active ingredient which is 6-methyl-5-hepten-2-one in the preparation of an inhibitory formulation for the treatment of a subject for inhibiting the activity of a haematophagous arthropod against a subject.
2. The use as claimed in claim 1 wherein the 6-methyl-5-hepten-2-one and geranylacetone of the composition are present in a weight ratio of at least or equal to about 0.5:1; 1:1; 1.5:1; 2:1; 20:1 or 200:1.
3. The use as claimed in claim 2 wherein the 6-methyl-5-hepten-2-one and geranylacetone are present in a weight ratio of about 1:1.
4. The use as claimed in claim 1 or claim 2, wherein the composition comprises octanal as a further active ingredient.
5. The use as claimed in any one of claims 1 to 4, wherein the composition consists essentially of geranylacetone in combination with the other active ingredients.
6. The use as claimed in any one of claims 1 to 3, wherein the composition comprises:
geranylacetone: 0.00001 - 10% ; 0.001 - 10% ; 0.01 - 10% ; 0.1 - 5%, or about 1%, and
6-methyl-5-hepten-2-one: 0.00001 - 10%, 0.1 - 10%; 0.1 - 5%; or about 1%.
7. The use as claimed in claim 6, wherein the composition comprises octanal: 0.001 - 0.1%, 0.005 - 0.05%, or about 0.01%.
8. The use as claimed in claim 7, wherein the composition comprises:
geranylacetone: 0.001% to 1% and 6-methyl-5-hepten-2-one: 0.001% and 1% .
9. The use as claimed in any one of claims 1 to 7, wherein the composition further comprises an active ingredient selected from the list consisting of: nonanal; at 0.1 - 10%; decanal at 0.0001 - 10%.
10. The use as claimed in any one of claims 1 to 9, wherein the composition further comprises an active ingredient selected from the list consisting of: benzaldehyde; naphthalene; acetophenone; (E)-2-octenal.
11. The use as claimed in any one of claims 1 to 10, wherein the composition comprises geranylacetone plus at least two, three or four of the further active ingredients.
12. The use as claimed in claim 1, wherein the composition comprises a mixture selected from the list consisting of; 1% 6-methyl-5-hepten-2-one, 0.01% octanal, 0.02% geranylacetone in ethanol; 1% 6-methyl-5-hepten-2-one, 1% geranylacetone, 0.01% octanal in ethanol; 1% 6-methyl-5-hepten-2-one, 0.0001% geranylacetone, 0.01% octanal in ethanol; 1% 6-methyl-5-hepten-2-one , 1% geranylacetone (in ethanol).
13. The use as claimed in any one of claims 1 to 12 wherein the subject is a human subject.

14. The use as claimed in any one of claims 1 to 13 wherein the haematophagous arthropod is an insect pest is the family Ceratopogonidae or family Culicidae or is a tick.
15. The use as claimed in any one of claims 1 to 14 wherein the haematophagous arthropod is selected from the list consisting of: members of the *Culex pipiens* complex; *Aedes aegypti*; *Anopheles stephensi*; *Anopheles gambiae* complex; *Culicoides impunctatus*; *Culicoides molestus*; *Lutzomyia* species; *Phlebotomine* species; Midges.
16. The use as claimed in any one of claims 1 to 15 wherein the composition is to be applied to achieve a topical dose of each of the active ingredient of between about 0.0025 $\mu\text{g}/\text{cm}^2$ to 100 $\mu\text{g}/\text{cm}^2$.
17. A formulation for treatment of a subject for inhibiting the activity of a haematophagous arthropod against the subject, which formulation is an ointment, solid or solution adapted for topical application, and which comprises:
 - (i) a dermatologically acceptable carrier; and
 - (ii) a composition comprising geranylacetone as an active ingredient plus a further active ingredient which is 6-methyl-5-hepten-2-one.
18. A formulation as claimed in claim 17 wherein the composition is as defined in any one of claims 2 to 12.
19. A formulation as claimed in claim 18 which comprises one or more volatile silicon fluids.
20. An article of manufacture comprising:
 - (i) a topical formulation as claimed in any one of claims 17 to 19,
 - (ii) aerosol or other spray means for administering the formulation topically.
21. An article of manufacture comprising:
 - (i) a topical formulation as claimed in any one of claims 17 to 19,
 - ii) instructions for use of the formulation against the haematophagous arthropod.
22. An article of manufacture for inhibiting the activity of a haematophagous arthropod against a subject,

which the article is for wearing by or otherwise protecting the subject,

wherein the article comprises a composition which comprises geranylacetone as an active ingredient plus a further active ingredient which is 6-methyl-5-hepten-2-one.
23. An article as claimed in claim 22 wherein the composition is as defined in any one of claims 2 to 12.
24. A method of repelling a haematophagous arthropod from an environment or preventing attraction to an environment,

the method comprising applying to the environment a composition which comprises geranylacetone as an active ingredient plus a further active ingredient which is 6-methyl-5-hepten-2-one.
25. A method as claimed in claim 24 wherein the composition is as defined in any one of claims 2 to 12.

26. A dispenser for repelling a haematophagous arthropod from an environment, the dispenser comprising a composition which comprises geranylacetone as an active ingredient plus a further active ingredient which is 6-methyl-5-hepten-2-one.
27. A dispenser as claimed in claim 26 wherein the composition is as defined in any one of claims 2 to 12.
28. A dispenser as claimed in claim 26 or claim 27 which is selected from the list consisting of: a candle, a coil, a slow release dispenser, an oil burner, an electric diffuser.
29. A dispenser as claimed in claim 26 or claim 27 which is an electrostatic chemical dispenser.
30. A dispenser as claimed in claim 29, wherein the electrostatic chemical dispenser comprises
- a) a conduit that contains liquid to be atomised, which liquid comprises, consists or consists essentially of the composition;
 - b) a spray electrode; and,
 - c) a discharge electrode.
31. A method of dispensing an insect repellent into an environment, which method comprises providing a dispenser as claimed in any one of claims 26 to 30 into said environment such that the composition is dispensed therefrom.
32. A use, article or dispenser as claimed in any of the preceding claims wherein an active ingredient is in the form of a precursor.