

PATENT SPECIFICATION

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(54) IMPROVEMENTS IN OR RELATING TO METHODS OF TREATING WATER CATCHMENTS

(71) I, THE SECRETARY OF STATE FOR DEFENCE, London, do hereby declare the invention, for which I pray that a patent may be granted to me, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The invention relates to a method of treating water catchments to reduce the ability of the catchments to act as mosquito breeding areas and to compositions and apparatus for carrying out the said method.

A high proportion of illness in tropical and sub-tropical countries is transmitted by mosquito species, particularly the malaria-carrying species such as *Anopheles (Cellia) Stephensi* (Liston), *Aedes (Stegomyia) aegypti* (Linnaeus) and *Culex quinquefasciatus*. Considerable effort has been devoted over many years to research into the mosquito life cycle and physiology leading to the treatment of water catchments, which constitute the sole breeding areas, with a systematic programme of insecticidal spraying. Although such schemes have produced great improvements, there are many difficulties in achieving long-term control of the mosquito population. In particular, it has been found that mosquito strains develop which are genetically resistant to the commonly used insecticides; and that the treated water catchment becomes polluted by the treatment and cannot be used for drinking and irrigation purposes. The latter aspect is particularly inhibiting since significant numbers of mosquitos are believed to breed in small water catchments such as rain-water butts which are required specifically for drinking and irrigation.

The present invention is derived from the observation that the larval and pupal stages of a mosquito's development, which stages always live submerged in a suitable water catchment, nevertheless require atmospheric oxygen for survival and eventual emergence of the adult from the pupa. Since this vital oxygen requirement is obtained by respiration across the air/water interface, several

attempts have been made to modify the characteristics of the interface, principally with soluble surface active agents. Some success has been obtained in this way in reducing the rate of emergence of adult mosquitoes, but the techniques are unacceptable because the water is effectively polluted.

The present invention aims to provide a method of treating the surface of a water catchment with product derived from natural sources which is non-toxic, biologically degradable, effective in very low concentrations and which can result, under ideal conditions, in a complete kill of mosquito pupae attempting to respire through the treated surface of a water catchment.

According to the present invention, a method of reducing the ability of a water catchment to act as a mosquito breeding area comprises forming upon the surface of the catchment at least one monolayer of a lipid, so that mosquito pupae are prevented from respiring by the surface pressure of the monolayer and thereby killed.

We have found that the lipids, particularly phospholipids, can be extremely effective in methods in accordance with the invention largely because of the very high surface pressure which is exerted by a monolayer of the material. In general, we have shown that a surface pressure of at least 40 dynes per square centimetre at 27° C and preferably at least 44 dynes per square centimetre is required to prevent the emergence of a mosquito pupa's respiratory trumpet through the monolayer. The quantity of lipid required is extremely low and theoretically the quantity need to be sufficient only to provide one lipid molecule for each area of water catchment surface having the area of a typical lipid hydrocarbon chain i.e. roughly one molecule per 60 square Angstroms of surface. In practice, 10–20 grammes of lipid per acre of water catchment surface should be adequate to achieve an effective monolayer, but of course more material may be desirable to ensure the maintenance of a substantially unbroken monolayer if the sur-

face is broken up by reeds, passing ships or wave motion. It is clearly preferable to use lipid which forms a liquid monolayer rather than a solid monolayer since the former will readily reform after breakage due to such causes and, for example, heavy rain.

We are aware that it has been reported that monolayers of certain long chain fatty alcohols, particularly lauryl alcohol, are effective inhibitors of pupal respiration, but using pure lauryl alcohol we have been unable to confirm these results. Additionally, of course, lauryl alcohol is not readily biologically degradable and is toxic to some forms of plant and animal life.

Readily available commercial phospholipids include phosphatidyl cholines such as egg lecithin and brain lipid extract, but in general may be any lipid of lipid-containing material selected from the classes comprising complex phospho-lipids, that is, the lecithins, cephalins and sphingomyelins which give monolayers with a surface pressure of at least 40 dynes per centimetre at 27° C. Other suitable phospholipids include glycerophosphatidyl glycerols and possibly other glyco-lipids.

In theory, the lipids may be provided as a monolayer on the surface of a water catchment of any size merely by depositing a sufficient quantity of the bulk lipid at one position on the surface and allowing the resulting surface pressure forces to spread a monolayer from this position. In practice, however, it is found that complete monolayers are formed more reliably by providing sources of lipid at a plurality of positions on a water catchment surface and apparatus for achieving this conveniently is desirable. According to an optional aspect of the present invention, therefore, apparatus for reducing the ability of a water catchment to act as a mosquito breeding area comprises a container housing a charge including at least one lipid and having means for applying the charge at a controlled rate to the surface of a water catchment. The design of suitable apparatus depends upon the physical state of the charge but will normally take the form of a powder or aerosol dispenser in which the means for dispersing the charge at a controlled rate are constituted by a supply of gas under pressure within or connected to the housing to force the charge through the delivery orifice by operation of a manually-operated outlet valve.

The coherent, tacky, solid, or viscous liquid forms in which the lipids exist at the ambient temperatures, typically 25–30° C, encountered in countries supporting disease carrying mosquito populations, are not readily dispersed *per se*, neither by the aforesaid apparatus nor by other methods. In accordance with an optional feature of the invention, therefore, the selected lipid is dispersed upon a particulate solid support or within a

suitable liquid medium to give materials which can be readily handled and readily discharged at a controlled rate, particularly from the aforementioned apparatus. Suitable particulate solids include finely-divided inert powders including purified particulate silicas, diatomaceous earth, powdered talc, fine sand, solid particulate polymers, particulate ion-exchange resins and other materials exerting a reversible sorptive action upon the lipid (that is, the lipid may be sorbed onto, and subsequently desorbed from the solid). It can be beneficial to provide solid particulate supports which have a specific gravity less than the treated water so that the particulate support floats upon the surface and releases the maximum amount of available sorbed lipid. This is particularly true where the support releases lipid over a considerable period of time which characteristic is desirable to maintain the lipid monolayer at maximum effectiveness for considerable periods. The particulate supporting material normally should be non-toxic to preserve a major advantage of the use of lipids in accordance with the invention, but it may sometimes be desirable to use as a support a material having some additional beneficial effect on the water catchment surface, for example to reduce evaporation. Similarly, it may be particularly advantageous to incorporate in the lipid film an attractant material which can lure adult mosquitos to lay eggs in the treated catchment.

Suitable liquid media in which the lipids may be dispersed include those lipid solvents which are substantially non-toxic when applied to the surface of a water catchment. If the lipid solvents are sufficiently volatile, of course, there is unlikely to be any significant toxic effect before evaporation of the solvent from the surface is complete. Particularly suitable lipid solvents are paraffin fractions such as hexane, kerosene and halogenated hydrocarbons such as chloroform, carbon tetrachloride, trichlorethylene and methyl dichloride. To increase solubility a proportion, generally 5–30% by volume, of a hydrophilic solvent such as methanol and isopropanol may be used.

The lipids are of course naturally occurring materials which degrade on storage and exposure to the atmosphere and it is very desirable to incorporate an antioxidant to reduce this. In accordance with another optional aspect of the invention, therefore, there is provided for carrying out a method in accordance with the invention, a composition which comprises at least one lipid dispersed upon a particulate solid support or in a liquid medium in combination with an effective amount of an antioxidant which reduces the rate of degradation of the lipid. Suitable antioxidants are those which do not interfere significantly with the surface pressure properties of the lipid and which are substantially non-toxic in the concentrations

at which the compositions are applied by methods in accordance with the invention. Typically, suitable antioxidants are of the well-known phenolic type such as butylated hydroxy toluene (BHT) butylated hydroxy anisole (BHA), propyl gallate and dodecyl gallate used in proportions generally from 0.01 to about 5% by weight based on the lipid.

In accordance with a further optional feature of the invention there are provided compositions comprising a lipid and an effective amount of antioxidant therefore both sorbed upon a particulate solid support and compressed into tablet form. The proportion of lipid in the composition preferably will be the maximum quantity which can be readily sorbed upon the particulate support and generally will be between about 1% and 20% by weight of the composition. Compositions in tablet, or other dosage, forms are particularly convenient to use in treating small water catchments such as rain-water butts. Thus, for example, in a mosquito control programme an appropriate dosage can be provided for treating the domestic water catchments of each individual inhabitant in a selected town or village.

The effectiveness of methods and apparatus in accordance with the invention will now be illustrated by the reference to the drawings filed with the provisional specification in which:—

Figure 1 is a histogram which shows the effectiveness of the phospholipids of the invention compared with a variety of apparently related materials;

Figure 2 is a cross-sectional view of an apparatus suitable for applying solutions or dispersions of lipids in accordance with the present invention, and

Figure 3 is a cross-sectional view of apparatus suitable for applying lipids which are sorbed on to particulate solids in accordance with the present invention.

In a series of trials, a simulated water catchment was provided having a surface area of approximately 100 square centimetres and containing pure water. A known number of active mosquito pupae (*Anopheles (Cellia) Stephensi*) totaling between 30 and 40 were introduced into the catchment and a 10% (i.e. 10 grms/100 ml) solution of a selected test material in chloroform was applied to the surface by the apparatus described with reference to Figure 2 in an amount sufficient to give a surface concentration of test material of at least 10^{-6} g/cm². The effectiveness of three typical phospholipids in accordance with the invention [all phosphatidylcholines and respectively brain lipid extract "Inmol" BS (Cambridge Biochemicals Ltd.) "Inmol G" (Cambridge Biochemicals Ltd.) and egg lecithin] compared with insoluble monolayers of materials having similar and lower equilibrium spreading pressure at a constant temperature

of 27° C is demonstrated in Figure 1. It is clearly seen that a complete kill of pupae was obtained using the phospholipids of the invention equal with the effectiveness of a glass plate placed upon the water catchment surface to induce complete anoxia of the pupae. As a control, distilled water having a surface tension of 72 dynes/cm is included in Figure 1.

Referring to Figure 2, apparatus suitable for applying solutions or dispersions of phospholipids to water catchment surfaces comprises a container 1 containing a supply of gas under pressure which can be released by a manually operated valve 2 into a venturi 3 and thereby withdraw a supply of phospholipid solution or dispersion 4 from housing 5 through tube 6. The resulting lipid-containing aerosol emerges from nozzle 7 and is readily directed in a controlled manner upon the surface of a water catchment.

Referring to Figure 3, apparatus suitable for applying phospholipids which are sorbed on to particulate solids comprises a housing 1 containing sorbed phospholipid material 2 feeding into the path of a stream of gas under pressure emerging from outlet 3. The resulting dispersion of particulate solid and gas emerges through nozzle 4 and can be applied in a ready controlled manner to a water catchment surface by operation of a manually controlled valve 5 downstream of a supply of compressed gas (not shown).

WHAT I CLAIM IS:—

1. A method of reducing the ability of a water catchment to act as a mosquito breeding area comprising forming upon the surface of the catchment at least one monolayer of a lipid. 100
2. A method according to Claim 1 wherein the lipid is a phospholipid. 105
3. A method according to Claim 2 wherein the phospholipid is a lecithin, a cephalin, a sphingomyelin, a glycerophosphatidylglycerol or a glyco-lipid. 110
4. A method according to claim 3 wherein the phospholipid is egg lecithin or brain lipid extract.
5. A method according to any one of Claims 1 to 4 wherein the surface pressure exerted by the lipid is at least 40 dynes per square centimetre at 27° C. 115
6. A method according to any one of Claims 1 to 5 wherein the concentration of lipid is in the range 10 to 20 grammes per acre of water catchment surface. 120
7. A method according to any one of Claims 1 to 6 wherein the lipid forms a liquid monolayer.
8. A method according to any one of Claims 1 to 6 wherein the lipid is dispersed upon a particulate solid support. 125
9. A method according to Claim 8 wherein



at which the compositions are applied by methods in accordance with the invention. Typically, suitable antioxidants are of the well-known phenolic type such as butylated hydroxy toluene (BHT) butylated hydroxy anisole (BHA), propyl gallate and dodecyl gallate used in proportions generally from 0.01 to about 5% by weight based on the lipid.

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WHAT I CLAIM IS:—

1. A method of reducing the ability of a water catchment to act as a mosquito breeding area comprising forming upon the surface of the catchment at least one monolayer of a lipid.

2. A method according to Claim 1 wherein the lipid is a phospholipid.

3. A method according to Claim 2 wherein the phospholipid is a lecithin, a cephalin, a sphingomyelin, a glycerophosphatidylglycerol or a glyco-lipid.

4. A method according to claim 3 wherein the phospholipid is egg lecithin or brain lipid extract.

5. A method according to any one of Claims 1 to 4 wherein the surface pressure exerted by the lipid is at least 40 dynes per square centimetre at 27° C.

6. A method according to any one of Claims 1 to 5 wherein the concentration of lipid is in the range 10 to 20 grammes per acre of water catchment surface.

7. A method according to any one of Claims 1 to 6 wherein the lipid forms a liquid monolayer.

8. A method according to any one of Claims 1 to 6 wherein the lipid is dispersed upon a particulate solid support.

9. A method according to Claim 8 wherein



the solid support is a finely divided inert powder.

10. A method according to Claim 9 wherein the powder is a purified particulate silica, a diatomaceous earth, a powdered talc, a fine sand, a solid particulate polymer, a particulate ion-exchange resin or a material having a reversible sorptive action upon the lipid.

11. A method according to Claim 9 or Claim 10 wherein the powder has a specific gravity less than the treated water.

12. A method according to any one of Claims 1 to 8 wherein the lipid is dispersed within a liquid medium.

13. A method according to Claim 12 wherein the liquid medium is a paraffin or a halogenated hydrocarbon.

14. A method according to Claim 13 wherein the liquid medium is hexane, kerosene, chloroform, carbon tetrachloride, trichlorethylene or methylene dichloride.

15. A method according to any one of Claims 12 to 14 wherein a proportion of a hydrophobic solvent is added to the liquid medium.

16. A method according to Claim 15 wherein the solvent is methanol or isopropanol.

17. A method according to Claim 15 or Claim 16 wherein the proportion is in the range 5 to 30 per cent by volume.

18. A method according to any one of Claims 8 to 14 wherein an antioxidant is added to the lipid.

19. A method according to Claim 18 wherein the antioxidant is butylated hydroxytoluene, butylated hydroxy anisole, propylgallate, or dodecylgallate.

20. A method according to Claim 19 wherein the antioxidant is added in proportions from 0.01 to about 5 per cent by weight based on the lipid.

21. A composition for use in a method according to any preceding claim comprising a lipid and an effective amount of an antioxidant both sorbed upon a particulate solid support and compressed into tablet form.

22. A method according to Claim 1 substantially as described in the specification.

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