LARVICIDAL OIL
WITHOUT INSECTICIDE

Full specification WHO/SIF/23.R1
Revised 10 December 1999

1. Specification

1.1 Description

The material shall consist of a mineral oil in the form of a homogeneous mobile liquid, free from dirt, water, and other visible extraneous matter. It may, if so specified, have additives incorporated to improve its physical performance. At the rates ordinarily used, it must not be toxic to fish, domestic animals, man, or plant life.

1.2 Chemical and physical requirements

The material, sampled from any part of the consignment (see method WHO/M/1.R1), shall comply with the requirements of section 1.1 and with the following requirements:

	Minimum	Maximum
Relative density at 30°C/30°C (section 2.1)		0.940
Distillation (section 2.2), proportion distilling at 200°C		50 mL/L
Flashpoint (section 2.3)	> 65°C	
Kinematic viscosity at 21.1°C (section 2.4)		1×10^{-5} m^2/s
Spreading pressure (section 2.5):		
Grade 1	4.6×10^{-2} N/m	
Grade 2	2.5×10^{-2} N/m	
Grade 3	1.8×10^{-2} N/m	
Stability of film (section 2.6)	2 hours	
Material soluble in water and oil layers (section 2.7)		25 mL/L
Toxicity to mosquito larvae (section 2.8):		
Anopheles stephensi, kill at 25°C	90%	
Aedes aegypti, kill at 25°C	75%	

1.3 Packing and marking of packages

The larvicidal oil shall be packed in suitable, clean containers, as specified in the order. All packages shall bear, durably and legibly marked on the container, the following:

Manufacturer's name
Larvicidal oil
Batch or reference number, and date of test

Net weight of contents
Date of formulation
Instruction for use

and the following minimum cautionary notice:

Keep well away from foodstuffs and animal feed and their containers.

2. Methods of determining chemical and physical properties

2.1 *Relative density*

Determine the relative density at 30°C/30°C by the CIPAC method MT 3.2 (CIPAC Handbook F, p.13).

2.2 *Distillation*

Determine the volume of the sample that distils at 200°C using the method described by the Institute of Petroleum (Standard IP 123/58)[1] and the American Society for Testing Materials (Standard D 158-54)[2] or any other equivalent standard method.

2.3 *Flashpoint*

Determine the flashpoint by the Tag closed tester CIPAC method MT 12.2 (CIPAC Handbook F, p.37).

2.4 *Kinematic viscosity*

Determine the kinematic viscosity at 21.1°C using one of the relevant methods described by the British Standards Institution (BS 188: 1957)[3] and the American Society for Testing Materials (Standard D445-53T)[4] or any other equivalent standard method.

2.5 *Spreading pressure*

2.5.1 *Standard solutions*

Solution 1 (spreading pressure 4.6 x 10^{-2} N/m). A 100 g/L solution of oleyl alcohol in medicinal paraffin.

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1 Institute of Petroleum. *Standard methods for testing petroleum and its products.* 13th ed., London, 1953.
2 American Society for Testing Materials. *Book of ASTM standards.* Philadelphia, 1958.
3 British Standards Institution. *Determination of the viscosity of liquids in c.g.s. units.* London, 1957 (BS 188: 1957).
4 American Society for Testing Materials. *Book of ASTM standards.* Philadelphia, 1958.

Solution 2 (spreading pressure 2.5 x 10^{-2} N/m). A 10 g/L solution of oleyl alcohol in medicinal paraffin.

Solution 3 (spreading pressure 1.8 x 10^{-2} N/m). A 10 g/L solution of terpineol in medicinal paraffin.

2.5.2 *Procedure*

Thoroughly clean a large glass funnel, not less than 20 cm in diameter, with chromic acid solution and remove all traces of acid by thorough washing with distilled water. Fix the funnel in a vertical position in a retort stand over a sink or receptacle to collect the water that overflows. Connect the stem to a water supply by means of rubber tubing. Turn on the water and allow it to overflow in order to give a clean surface for testing. Turn off the water and tilt the funnel slightly to bring the water level to about 3 mm below the rim of the funnel.

Take a clean glass rod in each hand, dip one rod into the sample and the other into the standard solution corresponding to the grade of the larvicidal oil and deposit a drop from each of the rods simultaneously on the water surface. Observe the spreading of the oil on the water surface. It is often easier to see the films if they are viewed from the level of the water surface.

The funnel must be cleaned between tests by allowing the water to overflow, or, if necessary, by cleaning with chromic acid solution, to ensure that the water surface used is not contaminated.

2.5.3 *Interpretation* of results

If the sample occupies more than half the surface, its spreading pressure is greater than that of the standard. If the sample and the standard solution occupy about equal areas, the spreading pressures are approximately equal. In these two instances, the sample is acceptable from the point of view of spreading pressure. If the standard solution occupies more than half the surface, the spreading pressure of the sample is lower than that of the standard. In this case the sample is unsatisfactory and unacceptable.

Only initial observations should be recorded. The standard solution may subsequently occupy a smaller area than when first allowed to spread on the surface, on account of the solubility of the spreading agent in water.

The test should be repeated especially when "borderline" oils are under examination.

2.6 Stability of film

2.6.1 *Apparatus*

Test-bowl of china or enamelled iron, 30 cm ± 1 cm in diameter.

2.6.2 *Procedure*

Thoroughly clean the test-bowl with light petroleum and then with chromic acid solution. Rinse thoroughly, first with hot distilled water and then with acetone, and finally dry. Thereafter, do not touch the inside of the bowl.

Fill the bowl almost completely with distilled water. Pipette 0.8-1.0 mL of the sample gently on to the surface of the water so that a complete film is formed extending to the edge of the bowl. The film must remain uniform and unbroken for at least 2 hours.

2.7 Material soluble in water and oil layers

2.7.1 *Apparatus*

Graduated cylinder, 100 mL capacity, with 0.2 mL graduations, fitted with a ground-glass stopper.

2.7.2 *Procedure*

Measure accurately 50 mL of the sample and 50 mL of distilled water into the cylinder at room temperature. Shake the mixture vigorously for 10 minutes so that thorough mixing of the two layers occurs. Then leave the cylinder undisturbed for 24 hours at room temperature.

Express any reduction in volume of either layer as a percentage of the total volume of sample taken.

2.8 Toxicity test for larvicidal oils

2.8.1 *Apparatus*

1. Glass beakers 400 mL in capacity and 7.3 cm in internal diameter.

2. Enamel rearing pans, about 40 cm by 36 cm.

3. A colony cage of wood or other suitable material, for rearing adult mosquitoes, provided in front with a wire screen and a cloth sleeve entrance, and large

enough to allow the mosquitoes sufficient space for swarming and mating: 60 cm cubical cages have been found satisfactory.

4. A small emergence cage for collecting the pupae.

5. A cage just large enough to confine a rabbit (or other suitable animal) inside the colony cage.

6. Pipettes for transferring larvae.

7. Pipettes for oil application.

2.8.2 *Standard colony of mosquitoes*

Obtain eggs from a known established colony, or place a gravid female mosquito in a 7.5 cm specimen tube, the mouth of which is plugged with moist filter paper, where oviposition usually takes place.

Transfer the eggs by means of a camel-hair brush to the rearing pans containing water maintained at $25^0C \pm 1^0C$. Feed the larvae with dry yeast powder or any other suitable food[1] sprinkled on the water surface every morning, increasing the quantity of food as the larvae grow. To avoid overcrowding of the larvae, do not keep more than 750 larvae in a pan. Change the water in the pans on alternate days, shaking the fresh water thoroughly in a bottle before pouring it into the pans. Collect the pupae as they are formed and place them in the emergence cage.

Transfer the hatched-out adults into the colony cage. Maintain a temperature of $24\text{-}27^0C$ and relative humidity of 75-80% inside the room and the colony cage (this is generally done by hanging wet cloths in the room and inside the colony cage). Keep cotton pads soaked in a 100 g/L glucose solution inside the cage for male mosquitoes to feed on. In order to provide the blood meal for female mosquitoes, introduce each night into the colony cage the small cage containing a rabbit (or other suitable animal) with shaved back[2]. Put an enamel bowl with water inside the colony cage for oviposition. Keep the cage in a room illuminated for about 9 hours a day by a 60 watt lamp and kept dark the rest of the time.

The colony must be tested periodically against standard larvicidal oil to ensure that it is behaving normally. A suitable standard is hexadecane.

2.8.3 *Procedure*

[1] Finely-powdered dog biscuit; powdered skimmed milk; a mixture of 1 part of dehydrated blood-serum and 2 parts of litmus milk; brewers' yeast; powdered dried toast or bread-crumbs; mature hay infusion (must be aerated vigorously every day); *Spirogyra*; or chopped flies.

[2] If a suitable animal is not available, the human hand may be introduced into the cage for short periods.

The test shall be performed at $25^0C \pm 1^0C$. Carry out 5 replicate tests simultaneously for each dosage of each test material.

Transfer 25 early 4th instar larvae[3] from the standard colony to each of fifteen 400 mL glass beakers containing 250 mL of distilled water. Pipette not more than 0.01 mL of the sample[4] gently on the surface of the water in each of 5 beakers and proceed similarly with doses of 0.02 mL and 0.04 mL, using 5 beakers for each.

The percentage mortality is recorded at the end of 24 hours. The test dosage of 0.01 mL is equivalent, in terms of field dosage, to approximately 24 litres per hectare.

2.8.4 *Recording of results*

The larvae shall be considered as dead if, at the end of 24 hours, they show no sign of swimming movements even after being gently touched with a glass rod. Report the average of the individual test results.

[3] The same test procedure may be used with pupae to evaluate the pupicidal effectiveness of oils.
[4] In instances where the mortality is high at the 0.01 mL level, the dosage should be reduced to 0.005 mL or lower for the precise determination of relative effectiveness.

LARVICIDAL OIL
WITH ADDED INSECTICIDE

Full Specification WHO/SIF/24.R1
Revised 10 December 1999

1. Specification

1.1 Description

The material shall consist essentially of a solution of a specified insecticide[1] in a mineral oil, in the form of a homogeneous mobile liquid, free from dirt, water, and other visible extraneous matter. It may, if so specified, have additives incorporated to improve its physical performance. At the rates ordinarily used, it must not be toxic to fish, domestic animals, man, or plant life. The technical insecticide and any additives used in the manufacture of the larvicidal oil shall comply with the requirements of the current approved specifications, where such specifications exist.

1.2 Chemical and physical requirements

The material, sampled from any part of the consignment (see method WHO/M/1.R1), shall comply with the requirements of section 1.1 and with the following requirements:

	Minimum	Maximum
Relative density at 30°C/30°C (section 2.1)		0.940
Distillation (section 2.2), proportion distilling at 200°C		50 mL/L
Flashpoint (section 2.3)	> 65°C	
Kinematic viscosity at 21.1°C (section 2.4)		1×10^{-5} m^2/s
Spreading pressure (section 2.5):		
Grade 1	4.6×10^{-2} N/m	
Grade 2	2.5×10^{-2} N/m	
Grade 3	1.8×10^{-2} N/m	
Stability of film (section 2.6)	2 hours	
Material soluble in water and oil layers (section 2.7)		25 mL/L
Toxicity to mosquito larvae (section 2.8):		

[1] The nature and content of the insecticide shall be agreed between the purchaser and manufacturer at the time of placing the order.

Anopheles *stephensi*, kill at 25°C	100%
Aedes aegypti, kill at 25°C	100%
Insecticide content on g/kg basis (section 2.9)	Within ± 5% of declared content
Average of all samples	Not less than declared content

1.3 Packing and marking of packages

The larvicidal oil shall be packed in suitable, clean containers, as specified in the order.

All packages shall bear, durably and legibly marked on the container, the following:

Manufacturer's name
Larvicidal oil to specification WHO/SIF/24.R1
Insecticide added ... g/kg
Batch or reference number, and date of test
Net weight of contents
Date of formulation
Instruction for use

and a cautionary notice appropriate to the insecticide added[1].

2. Methods of determining chemical and physical properties

2.1 Relative density

Determine the relative density at 30°C/30°C by the CIPAC method MT 3.2 (CIPAC Handbook F, p.13).

2.2 Distillation

Determine the volume of the sample that distils at 200°C using the method described by the Institute of Petroleum (Standard IP 123/58)[2] and the American Society for Testing Materials (Standard D 158-54)[3] or any other equivalent standard method.

2.3 Flashpoint

Determine the flashpoint by the Tag closed tester CIPAC method MT 12.2 (CIPAC Handbook F, p.37).

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1 For the wording of the notice see the specification for the corresponding technical product.
2 Institute of Petroleum. *Standard methods for testing petroleum and its products*, 13th ed., London, 1953.
3 American Society for Testing Materials. *Book of ASTM standards*, Philadelphia, 1958.

2.4 Kinematic viscosity

Determine the kinematic viscosity at 21.1°C using one of the relevant methods described by the British Standards Institution (BS 188: 1957)[1] and the American Society for Testing Materials (Standard D 445-53T)[2] or any other equivalent standard method.

2.5 Spreading pressure

2.5.1 *Standard solutions*

Solution 1 (spreading pressure 4.6 x 10^{-2} N/m). A 100 g/L solution of oleyl alcohol in medicinal paraffin.

Solution 2 (spreading pressure 2.5 x 10^{-2} N/m). A 10 g/L solution of oleyl alcohol in medicinal paraffin.

Solution 3 (spreading pressure 1.8 x 10^{-2} N/m). A 10 g/L solution of terpineol in medicinal paraffin.

2.5.2 *Procedure*

Thoroughly clean a large glass funnel, not less than 20 cm in diameter, with chromic acid solution and remove all traces of acid by thorough washing with distilled water. Fix the funnel in a vertical position in a retort stand over a sink or receptacle to collect the water that overflows. Connect the stem to a water supply by means of rubber tubing. Turn on the water and allow it to overflow in order to give a clean surface for testing. Turn off the water and tilt the funnel slightly to bring the water level to about 3 mm below the rim of the funnel.

Take a clean glass rod in each hand, dip one rod into the sample and the other into the standard solution corresponding to the grade of the larvicidal oil, and deposit a drop from each of the rods simultaneously on the water surface. Observe the spreading of the oils on the water surface. It is often easier to see the films if they are viewed from the level of the water surface.

The funnel must be cleaned between tests by allowing the water to overflow, or, if necessary, by cleaning with chromic acid solution, to ensure that the water surface used is not contaminated.

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[1] British Standards Institution. *Determination of the viscosity of liquids in c.g.s. units.* London, 1957 (BS 188: 1957).

[2] American Society for Testing Materials. *Book of ASTM standards*, Philadelphia, 1958.

2.5.3 *Interpretation of results*

If the sample occupies more than half the surface, its spreading pressure is greater than that of the standard. If the sample and the standard solution occupy about equal areas, the spreading pressures are approximately equal. In these two instances, the sample is acceptable from the point of view of spreading pressure. If the standard solution occupies more than half the surface, the spreading pressure of the sample is lower than that of the standard. In this case, the sample is unsatisfactory and unacceptable.

Only initial observations should be recorded. The standard solution may subsequently occupy a smaller area than when first allowed to spread on the surface, on account of the solubility of the spreading agent in water.

The test should be repeated especially when "borderline" oils are under examination.

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2.6.2 *Procedure*

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Fill the bowl almost completely with distilled water. Pipette 0.8-1.0 mL of the sample gently on to the surface of the water so that a complete film is formed extending to the rim of the bowl. The film must remain uniform and unbroken for at least 2 hours.

2.7 Material soluble in water and oil layers

2.7.1 *Apparatus*

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2.7.2 *Procedure*

Measure accurately 50 mL of the sample and 50 mL of distilled water into the cylinder at room temperature. Shake the mixture vigorously for 10 minutes so that thorough mixing of the two layers occurs. Then leave the cylinder undisturbed for 24 hours at room temperature. Express any reduction in volume of either layer as a percentage of the total volume of sample taken.

2.8 Toxicity test for larvicidal oils

2.8.1 *Apparatus*

1. Glass beakers 400 mL in capacity and 7.3 cm in internal diameter.

2 Enamel rearing pans, about 40 cm by 36 cm.

3. A colony cage of wood or other suitable material, for rearing adult mosquitoes, provided in front with a wire screen and a cloth sleeve entrance, and large enough to allow the mosquitoes sufficient space for swarming and mating: 60 cm cubical cages have been found satisfactory.

4. A small emergence cage for collecting the pupae.

5. A cage just large enough to confine a rabbit (or other suitable animal) inside the colony cage.

6. Pipettes for transferring larvae.

7. Pipettes for oil application.

2.8.2 *Standard colony of mosquitoes*

Obtain eggs from a known established colony, or place a gravid female mosquito in a 7.5 cm specimen tube, the mouth of which is plugged with moist filter paper, where oviposition usually takes place.

Transfer the eggs by means of a camel-hair brush to the rearing pans containing water maintained at $25^0C \pm 1^0C$. Feed the larvae with dry yeast powder or any other suitable food[1] sprinkled on the water surface every morning, increasing the quantity of food as the larvae grow. To avoid overcrowding of the larvae, do not keep more than 750 larvae in a pan. Change the water in the pans on alternate days, shaking the fresh water thoroughly in a bottle before pouring it into the pans. Collect the pupae as they are formed and place them in the emergence cage.

Transfer the hatched-out adults into the colony cage. Maintain a temperature of $24\text{-}27^0C$ and relative humidity of 75-80% inside the room and the colony cage (this is generally done by hanging wet cloths in the room and inside the colony cage). Keep cotton pads

[1] Finely-powdered dog biscuit; powdered skimmed milk; a mixture of 1 part of dehydrated blood-serum and 2 parts of litmus milk; brewers' yeast; powdered dried toast or bread-crumbs; mature hay infusion (must be aerated vigorously every day); *Spirogyra*; or chopped flies.

soaked in a 100 g/L glucose solution inside the cage for male mosquitoes to feed on. In order to provide the blood meal for female mosquitoes, introduce each night into the colony cage the small cage containing a rabbit (or other suitable animal) with shaved back[2]. Put an enamel bowl with water inside the colony cage for oviposition. Keep the cage in a room illuminated for about 9 hours a day by a 60 watt lamp and kept dark the rest of the time.

The colony must be tested periodically against a standard larvicidal oil to ensure that it is behaving normally. A suitable standard is hexadecane.

2.8.3. *Procedure*

The test shall be performed at $25^0C \pm 1^0C$. Carry out 5 replicate tests simultaneously for each dosage of each test material.

Transfer 25 early 4th instar larvae[3] from the standard colony to each of fifteen 400 mL glass beakers containing 250 mL of distilled water. Pipette not more than 0.01 mL of the sample[4] gently on the surface of the water in each of 5 beakers and proceed similarly with doses of 0.02 mL and 0.04 mL, using 5 beakers for each.

The percentage mortality is recorded at the end of 24 hours. The test dosage of 0.01 mL is equivalent, in terms of field dosage, to approximately 24 litres per hectare.

2.8.4 *Recording of results*

The larvae shall be considered as dead if, at the end of 24 hours, they show no sign of swimming movements even after being gently touched with a glass rod. Report the average of the individual test results.

2.8.5 *Insecticide content*

Determine the content of the added insecticide by the method included in the relevant specification. The method shall be agreed between the purchaser and manufacturer at the time of placing the order.

[2] If a suitable animal is not available, the human hand may be introduced into the cage for short periods.
[3] The same test procedure may be used with pupae to evaluate the pupicidal effectiveness of oils.
[4] In instances where the mortality is high at the 0.01 mL level, the dosage should be reduced to 0.005 mL or lower for the precise determination of relative effectiveness.