its insertion, for the intensity of microfilariae at this site reflects most truly the presence of adult worms situated above the waist and it is not influenced, as is the scapula snip, by microfilariae, at the limit of their upward spread, emanating from worms situated below the waist. It is also true that high counts at the deltoid are more commonly associated with microfilarial invasion of the eye than are high counts at the scapula.

Finally, the assessment of microfilarial densities in the eyes is made in the manner described above by ophthalmoscopic counts of the numbers in the two anterior chambers.

Division of labour in the team assessing microfilarial densities

To carry out the methods of assessing microfilarial densities described above a trained team of four persons is needed. The first man explains the procedure to the patient, prepares him for examination, cleans the skin with spirit prior to snipping, staunches the skin wounds and puts mydriatic drops in the patient's eye. The second man, who will usually be the medical officer, takes the skin snips and passes them on the needle to the third man, whose job is to weigh and record. It is also the duty of the man taking the skin snips to examine the eyes and count the numbers of microfilariae seen in the anterior chambers. The fourth man teases the snips and ensures that the slides remain wet for 10-15 minutes before being thoroughly dried and fixed for subsequent transport to the laboratory. If other aspects of the disease are being investigated simultaneously and other workers are present to assist, some modification of this routine may, of course, be necessary.

Conclusions

It is considered that the method described in this note will give the most accurate assessment attainable of microfilarial densities in populations surveyed for research purposes. It is one which can be carried out successfully under field conditions, and, if adequate numbers of people from all age-groups are surveyed in the different topographical environments where onchocerciasis is found, the method will give the maximum amount of information on the differences in microfilarial densities in such populations. The information so obtained may then be correlated with observations made at the same time on eye lesions, nodule distribution, nutritional status and genetically determined characteristics. Later it may be possible to correlate microfilarial densities with infective biting densities in the *Simulium* population.

Insecticidal Control of Filariasis *

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The ways in which chemicals are used to control the mosquito vectors of filariasis are somewhat conditioned by the species or genus concerned. Vectors of *Wuchereria malayi* in south-east Asia, which are members of the genus *Mansonia*, complete their larval development attached to aquatic weeds such as *Pistia* and *Eichhornia*. The principal vector of *W. bancrofti*, the tropical house mosquito *Culex fatigans*, develops most strongly in polluted waters close to urban conditions. However, on the Pacific islands bancroftian filariasis is transmitted by *Aëdes* mosquitos breeding in tree-holes and crab-holes. Meanwhile in forested areas in Malaya, Indonesia and West Africa, the local *Anopheles* are capable of acting as vectors of either type of filaria.

Adulticides

The house-spraying programme with wall deposits of DDT, so widely used against malaria, has proved inadequate to control the vectors of filariasis, even when applied at densities of 200 mg per square foot.[a] Antimalarial sprays have been reported to be much less effective against *Culex fatigans* than against *Anopheles darlingi* in British Guiana (Giglioli, 1948),

* Note submitted to the WHO Expert Committee on Filariasis, July 1961.

[a] A convenient conversion rate is 100 mg per square foot = 1 g per square metre.

A. maculatus in Malaya (Wharton, 1951), *A. gambiae* in Kenya (Davidson, 1953) and *Aëdes aegypti* in the Virgin Islands (Brown & Williams, 1949). *C. fatigans* is normally less DDT-susceptible than other mosquitos (Wharton, 1955), and wall deposits have the effect of driving them out of the treated huts, usually without killing them (Wharton, 1951; Reid & Wharton, 1956). Nevertheless, the DDT deposits do have some effect; in the Virgin Islands, a series of four treatments in two years reduced the infestation of *C. fatigans* by almost 60% and the parasite rate by about 50%, so that mosquitos with infective filaria larvae could no longer be found. However, repeated DDT residual sprays have led to the development of DDT-resistance in adult *C. fatigans* in different parts of the world, as for example in North India (Pal et al., 1952) and La Réunion (Hamon, 1953).

Residual deposits of γ-BHC were more effective, since this rapid-acting and partially fumigant insecticide kills the mosquitos before they leave the huts; moreover, deposits at the normal rate of application (25-40 mg per square foot) remained effective for at least three months (Wharton & Reid, 1950). Residual deposits of dieldrin at 50 mg per square foot were even better in that their effectiveness persisted for at least seven months (Davidson, 1953). However, dieldrin-resistance has subsequently developed in adult *C. fatigans* in parts of Malaya (Wharton, 1958b), in the Republic of Upper Volta (Hamon et al., 1958) and elsewhere. Dieldrin was recommended as the residual insecticide of choice for filariasis control in India, but dieldrin-resistance has frequently developed to the point where only heavy deposists of γ-BHC can effect control of *C. fatigans* (Nair [b]).

Experience with the *Mansonia* vectors of *W. malayi* has been similar. In Kerala, India, deposits of DDT at 200 mg per square foot were first reported to remain effective against *M. uniformis* and *M. annulifera* for 20 weeks (Singh et al., 1956), but later the protective period was found to be only six weeks (Krishnan et al., 1958). In Ceylon, deposits of DDT at 85 mg per square foot allowed infected *M. uniformis* to reappear three months later (Antonipulle et al., 1958). Dieldrin at 40 mg per square foot is applied against these vectors in Malaya at six-month intervals (Hassan, 1959). Yet dieldrin at 100 mg per square foot was found not to reduce the attack and infection by *M. longipalpis* in rural Malaya (Wharton, 1958a).

[b] In a report reviewed in *Trop. Dis. Bull.*, 1960, **57**, 930.

The vector of bancroftian filariasis in eastern Asia, *C. pipiens pallens*, has been controlled with DDT residual sprays and BHC fumigation in mainland China (Li, 1959), and by DDT and dieldrin wall sprays in southern Japan (Sasa et al., 1959). But the adult control was found to be transitory in Japan, so that larvicidal measures were added; moreover, resistance to DDT and dieldrin has lately been reported in Japanese populations of *C. p. pallens*. In Bombay State, the control of bancroftian filariasis required the simultaneous use of drugs (diethylcarbamazine: Hetrazan) as well as adulticides and larvicides (Patel & Paranjpey, 1958). Residual antimalarial sprays have reduced *M. uniformis* vector activities in the hills of Kerala, but not *C. fatigans* in the settlements of Assam. The increasing difficulties with residual deposits of chlorinated hydrocarbon insecticides for filariasis control are exemplified by the attempts made at Belém, Brazil, to control *C. fatigans* by residual sprays; the inadequacy of DDT at 200 mg per square foot led to the substitution of γ-BHC at 30 mg per square foot, and finally to dieldrin at 100 mg per square foot, which resulted in infestations three months later that were even higher than before (Rachou, 1957).

The first experiments with organophosphorus compounds as residual sprays for culicines were performed against *Mansonia* in Kerala; they indicated that malathion at 25 mg per square foot remained effective for one week and diazinon at the same dosage remained effective for three to four weeks (Krishnan et al., 1958). The most promising organophosphorus insecticide is Baytex (Compound 29493, Farbenfabriken Bayer A.G.); applied to mud walls at 100 mg per square foot in Léopoldville, it remained effective against *C. fatigans* for six months (Cerf & Lebrun, 1959). Laboratory experiments have proved Baytex to be the most long-lasting of all the organophosphorus insecticides tested against *Anopheles quadrimaculatus* (Labrecque et al., 1960). Field experiments against *A. albimanus* in El Salvador (Schoof, 1960; Communicable Disease Center, 1961) have shown deposits of Baytex at 200 mg per square foot to remain effective for 14 weeks; moreover, at these high dosage levels malathion was as effective as Baytex on thatch or wood, but not on mud walls.

Thus these organophosphorus compounds demand consideration as possible residual insecticides for filariasis control. However, malathion-resistance is now known in *C. fatigans* larvae at Douala, Cameroun (Mouchet et al., 1960). Pyrethrins still remain as a last resort. Weekly pyrethrin sprays proved effective against *C. fatigans* twenty years ago; it is now known that their residual effectiveness may be prolonged by adding the synergist piperonyl butoxide. Among the new group of carbamate insecticides, Sevin is quite effective as a residual spray, while Hercules AC-5727 has proved outstanding in residual effect against *A. quadrimaculatus* (Labrecque et al., 1960).

Larvicides

The larvae of *C. fatigans* were found to be susceptible to DDT applied in oil solution or emulsion, but the dosages required were two to three times that needed for *Aëdes* and *Anopheles* larvae (Ferguson et al., 1949), and thus of the order of 0.25-0.5 pound per acre.[c] They become more difficult to kill in polluted water (Hurlbut & Bohart, 1945), the mortality decreasing with increase in suspended solids (Parthasarathy & Krusé, 1954). In heavily contaminated water in Malaya, DDT dosages up to 2.0 pounds per acre were ineffective against *C. fatigans* larvae (Reid, 1956). Moreover, DDT-resistance has frequently developed as a result of continued DDT treatments, well-documented cases having been reported from Venezuela (Blazquez [d]) and Taiwan (Liu, 1958).

Oil solutions or emulsions of γ-BHC or dieldrin initially proved highly larvicidal for *C. fatigans*, treatments of 0.25 pound per acre causing almost complete mortality (Reid, 1956). Emulsion concentrates containing 20% of the insecticide are diluted with 30 parts of water and applied at approximately 4 Imperial gallons per acre.[e] Such γ-BHC larvicides have proved initially effective, but experience in India (Rajagopalan et al., 1954), Malaya (Reid, 1955), French Guiana (Floch & Fauran, 1958) and elsewhere has indicated the development of strong resistance. Dieldrin at 3 pounds per acre could at first achieve control of *C. fatigans* in latrine catch-pits in Java (Chow & Thevasagayam, 1957), but high dieldrin-resistance developed after only three to four weekly applications (Chow et al., 1959).

Resistance to γ-BHC and dieldrin alike has become extremely prevalent in parts of the world where these insecticides have been used against *C. fatigans*. It has also developed in larvae of the filariasis vectors *C. pipiens* in Egypt and *C. p. pallens* in Japan, as well as in *C. quinquefasciatus* (*fatigans*) in California.

In India and Malaya, the only reliable larvicide available for *C. fatigans* is a high-spreading unrefined oil applied at 25-40 Imperial gallons per acre (Reid, 1956; Nair [b]). Waste automobile oil could serve provided it is capable of spreading; precise investigations (Toms, 1950) indicate that a spreading pressure of 20 dynes/cm should be adequate, although 35 dynes/cm may be necessary for highly polluted water. A proprietary high-spreading oil is marketed under the name of Malariol HS (Shell Oil Company). It is considered unlikely that *C. fatigans* could develop resistance to this treatment, although oil resistance has been recorded to have developed in *Anopheles subpictus* in Ceylon (Jayewickreme, 1950).

Organophosphorus insecticides for control of culicine larvae resistant to the chlorinated hydrocarbons have been extensively developed in the United States of America. Parathion has been applied at 0.1 pound per acre in uninhabited agricultural areas, but its high toxic hazard is a problem. EPN has proved safe and effective at 0.075 pound per acre (Geib, 1955), but it is no longer available commercially. Malathion is safe and effective, but the high dosage required (0.5 pound per acre) makes its use expensive. Moreover, this dosage, although effective against *Aëdes* larvae, is insufficient to control *Culex* (Gjullin & Peters, 1956). The LC_{50} for normal *Culex fatigans* was found to be 4.8 p.p.m. for malathion as compared with 0.034 p.p.m. for parathion (Thevasagayam, 1957). In septic tanks, malathion has proved ineffective where EPN was highly effective (Roman et al., 1957). Subsequently malathion-resistance has developed in *Culex tarsalis* (Gjullin & Isaak, 1957) and in *C. fatigans* (Mouchet et al., 1960).

Water-soluble organophosphorus compounds, such as Phosdrin, Dipterex (Dylox), and its derived relative DDVP, have proved valuable for dispensing into irrigation water and are also effective in polluted water. Their insecticidal effect is soon destroyed, but this may actually be an advantage in water used for washing or drinking. More persistent organophosphorus larvicides include diazinon, Guthion and Ronnel, the last being without toxic hazard. But the most effective of this group are Dibrom and Baytex, either compound controlling saltmarsh *Aëdes* larvae at 0.05 pound per acre; Baytex is the best of all against *A. quadrimaculatus*. All these compounds may be formulated by adsorption on to fine granules, the best mineral carriers being diato-

[c] A convenient conversion rate is 1 pound per acre = 1120 g per hectare.

[d] Unpublished working document WHO/Insecticides/68 (1957).

[e] A convenient conversion rate is 1 Imperial gallon per acre = 11.23 litres per hectare.

mite, followed by prophyllite and attapulgite (Labrecque et al., 1956; Lewallen & Bryden, 1958). Malathion granules often prove more effective than liquid formulations.

A granular formulation suitable for distribution by hand has recently been developed by coating vermiculite with Paris green (Rogers & Rathburn, 1960). In a cement mixer, 53 pounds (24 kg) of vermiculite No. 4 are coated with 30 pounds (13.6 kg) of a mixture of one part Triton N-101 emulsifier and nine parts oil (e.g., Standard Oil No. 341), and treated with 17 pounds (7.7 kg) of Paris green (90% pure). Applied at 4 pounds per acre, these coarse granules have given excellent control of *C. quinquefasciatus* (*fatigans*) in Georgia and Florida (Fehn et al., 1959). Recently a formulation of 8% cupric metarsenite on vermiculite has been made available commercially (Air-Flo Green Granular: Chipman Chemical Company); since the particles are finer than with Paris green, they are more readily ingested by the larvae on release into the water. It is considered unlikely that resistance will develop against these arsenicals.

Effective control of culicine larvae may be always obtained, but at increased cost, with 0.1% pyrethrin emulsions. It has recently been found that the addition of 2% piperonyl butoxide to 0.2% pyrethrins allows water concentrations of 0.4 p.p.m. pyrethrins to remain insecticidal to *Aëdes aegypti* larvae for 14 weeks (Brooke, 1958).

Control of the larvae of the *Mansonia* vectors of *W. malayi* is a special case. *M. annulifera* has a preference for the water lettuce *Pistia stratiotes*, while *M. uniformis* prefers the water hyacinth *Eichhornia speciosa* (Burton, 1960); the aquatic plant *Colocasia antiquorum* is seldom attractive as a point of attachment for the larvae. *Mansonia* control in Ceylon and Kerala has been carried out by destroying the aquatic plants with the herbicide MCPA (Chow, 1953); this is applied as a 1.25% aqueous solution of its sodium salt at 40 Imperial gallons per acre (WHO Expert Committee on Insecticides, 1960). Control of the water fern *Salvinia auriculata* in Ceylon has been accomplished with pentachlorophenol (PCP); the 14% emulsion concentrate is diluted with 20 parts of water and the mixture applied at 130 Imperial gallons per acre (Chow et al., 1955). Recently the new herbicide Simazine has completely controlled *Pistia* at 6 pounds per acre with no adverse effect on fish, amphibians or *Colocasia*.

Insecticide granules can penetrate the vegetation sufficiently to treat the water for *Mansonia* control in overgrown ponds and tanks. Dieldrin applied in granules at 0.12 pound per acre achieved good control of *M. uniformis* under *Eichhornia* in Singapore (Dowling, 1955). EPN granules were more effective and faster acting than dieldrin granules against *M. indubitans* under *Pistia* in Florida (Chapman, 1955). It would appear that both water-soluble organophosphorus compounds and granules of the relatively insoluble organophosphorus compounds could be profitably assessed for *Mansonia* control.

On the islands of the Pacific, where the vectors of *W. bancrofti* are mainly *Aëdes* mosquitos breeding in small bodies of rainwater, dieldrin pellets have proved very effective. Each pellet weighs 10 g, and contains 16% γ-BHC or dieldrin incorporated in a mixture of sand and cement (Elliott, 1955); one pellet is suitable for two litres of water and prevents larval infestation for at least one year. Treatment of water-pots and holes at the bases of trees with these dieldrin pellets has given excellent control against the vectors *A. pseudoscutellaris* in Fiji (Burnett, 1960) and *A. polynesiensis* in the Tokelau Islands.[f] Piles of coconut shells may be sprayed with dieldrin suspensions, while the incidence of sporadic fallen and opened coconuts may be minimized by a programme of rat control (Bonnet & Chapman, 1958). Crab holes may be eliminated by plugging them and controlling the crabs with rice-bran baits containing γ-BHC or aldrin. Although *A. pseudoscutellaris* can develop DDT-resistance (Burnett, 1960), no dieldrin-resistance has yet appeared in these *Aëdes* vectors. From experience in Tahiti (March et al., 1960), it is considered that an effective programme of filariasis control on Pacific islands could be achieved with drug treatment supplemented by elimination or treatment of all breeding places within 100 m of any dwelling.

[f] Laird, M.—personal communication, 1961.

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