# DDT as a Larvicide against *Simulium*[1]

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The investigations here reported were undertaken in an attempt to devise a practical method for the control or eradication of several species of *Simulium* (black flies or buffalo gnats) which are believed responsible for the transmission of a serious human filarial disease caused by *Onchocerca volvulus* in Guatemala and southern Mexico. The species of *Simulium* thought to be most important are *S. metallicum* Bellardi 1859 and *S. ochraceum* Walker 1860. The larvae of *S. callidum* Dyar & Shannon 1927, *S. mexicanum* Bellardi 1862 and *S. exiguum* Roubaud 1906 as well as several as yet unidentified species were also abundant in the streams where tests were made, but we were not able to study the differential responses to treatment of the different species, if any. Most of the work was done at or near the town of Yepocapa in the Department of Chimaltenango, Republic of Guatemala during October and November 1944 and April and May 1945, but a few tests were run in streams near Pochuta in the same department and in the Rio Michatoya which drains Lake Amatitlan in the Department of Guatemala.

For the purpose of orienting those who may be unfamiliar with *Simulium* a brief summary of the life history of these flies is here given.

The adult *Simulium* flies are diurnal, but generally attack most readily in shaded or partially shaded situations. They rarely enter dwellings. The maximum flight range is unknown, but scattered observations seem to indicate that it is considerable, in some cases exceeding five kilometers. The eggs are laid in swift streams on stones or trailing leaves covered by a thin film of water. Patches of eggs in favorable places may contain several thousand eggs, the results of the efforts of a number of females. The length of the egg stage of the Guatemalan species is not accurately known. The larvae attach themselves to stones, sticks and submerged or trailing vegetation by means of a circlet of hooks on the posterior end. These hooks are fastened into a patch of silk spun by the larva. Larvae move from place to place by spinning long threads on which they let themselves down to new locations, or by spinning new patches of silk and transferring the posterior hooklets to them by a series of looping movements. The length of larval life for the various Guatemalan species is not accurately known. Respiration in the larvae takes place both through the skin and by means of anal gills, both air in the form of fine bubbles and dissolved oxygen being utilized. Food consists of the microscopic plant and animal life caught in the basket-like mouth brushes which are held open by the current. Pupation takes place in shoe or slipper-shaped silken cocoons attached to rocks, leaves, etc., the open end of the cocoon being downstream and the pupa being held in place by hooks. Respiration during the pupal stage is accomplished by means of branched filamentous gills protruding from the anterior end of the pupa. The length of the pupal stage for the Guatemalan species is unknown.

The actual investigation may be divided into two phases, first, preliminary studies to determine whether DDT is capable of killing *Simulium* larvae under the conditions obtaining in the onchocercosis zone, and second, development of economically practicable methods of application.

The preliminary work was done with a concentrate consisting of 60 per cent xylene, 20 per cent DDT, and 20 per cent *Triton x-100* by weight. This concentrate, when diluted with 4 parts of water, gives a stable emulsion containing 4 per cent DDT. The first tests were made in wooden sluice boxes where the flow of water and hence the dilution of DDT could be accurately determined. Vegetation with *Simulium* larvae attached was placed in the boxes and the emulsion poured in

[1] This investigation was carried out under the auspices of the Pan-American Sanitary Bureau and formed part of a cooperative program with the governments of Guatemala and Mexico for the eradication of Onchocerciasis. The senior author worked under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Gorgas Memorial Laboratory, Panama, R. de P. Expenses were partially defrayed by a grant in aid from the Office of the Coordinator of Inter-American Affairs.
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[Table 1.—Trials of 4 per cent DDT emulsion in sluice boxes.

| Box No. | Flow in Gals. Per Minute | Calculated ppm. | Estimated No. Larvae in Box | Larvae Attached At End of Treatment | Dead Larvae in Net | Pupae Collected | Pupae Emerged | Per Cent Emergence |
|---|---|---|---|---|---|---|---|---|
| 1 | 100 | 7.8 | few | none | 8 | 58 | 11 | 6.38 |
| 2 | 300 | 2.6 | many | fair no. | 3[1] | 44 | 0 | 0 |
| 3 | 80 | 9.9 | 100 | none | 327 | 29 | 0 | 0 |
| 4 | 260 | 3.0 | few | none | 76 | 0 | — | — |
| 5 | 200 | 3.9 | 300 | 3, bottom box | 103 | 59 | 7 | 4.13 |
| 6 | 220 | 3.6 | 100 | none | not counted | 10 | 0 | 0 |

[1] Strong current probably washed most larvae through net.

slowly at the up-stream end of the box. A cloth net was placed over the lower end of the sluice box to catch detaching larvae. All larvae found in nets were dead or moribund within 2 hours after the treatment. Each treatment consisted in the application of approximately 750 cc. of the emulsion over a period of 10 minutes. Table 1 gives the results of this series of tests.

The pupae mentioned in the table were taken from the vegetation in the sluice box subsequent to treatment and held in individual emergence tubes for a least 3 days. Emergence under the same conditions of pupae from untreated streams varies widely but is generally at least 25 per cent. We feel that DDT kills a large proportion of pupae but is probably less effective against this stage than against the larvae.

It was next considered advisable to test the emulsion on a large scale and at greater dilutions. For this purpose a number of streams and small rivers were surveyed, their flow estimated and the presence of adequate numbers of *Simulium* larvae determined. They were then treated by pouring in over a period of an hour a quantity of emulsion sufficient to give the desired concentration of DDT. In some cases sluice boxes containing vegetation bearing *Simulium* larvae were placed in the streams some distance below the point of application, while in others certain leaves, branches or rocks bearing many larvae before treatment were marked and checked subsequent to treatment. Checking was usually done 24 hours after treatment, occasionally 48 hours later Table 2 summarizes the results obtained in this series of tests.

As can be seen from table 2, excellent results were obtained at very high dilutions. The last test is especially noteworthy, as 100 per cent kill was obtained at an initial concentration of only 1 part DDT in nearly 10 million parts of water.

Having established that the DDT emulsion will give satisfactory kills at very low concentrations, it was decided to try to see what effect treatment of all streams over a relatively large area would have on the adult population. This experiment was conducted by the junior author with the assistance of Dr. H. Elishawitz, and is being reported in detail separately, but will be summarized here for the sake of completeness.

An area of approximately 20 sq. km. containing three relatively large rivers and 27 smaller tributary streams, and lying well within the Onchocerca zone, was selected. An attempt was made to select an area having a high *Simulium* population and one that was as well isolated as possible by natural barriers from adjacent *Simulium*-infected territory. Treatment consisted of application to the area of the 60-20-20 xylene-*Triton*-DDT mixture diluted with water to give a 4 per cent emulsion, the dosage being calculated to give 0.1 ppm initial concentration. A satisfactory kill was obtained.

To check the adult populations, biting rates on human subjects at six stations in the treated area and three stations in an adjacent untreated area were taken over a period of 7 weeks. The results were not as clear cut as could have been wished, as the test was apparently made at the beginning of a natural seasonal decline in the adult population. Since little information was available as to the seasonal variations in *Simulium* abundance, this complication could not have been anticipated. The

Table 2

| Test No. | Quantity 4 Per Cent Emulsion in Gals. | Flow in Gals. Per Minute | DDT ppm. | Distance of Observed Kill in Meters | Remarks |
|---|---|---|---|---|---|
| 1[1] | 4 | 600 | 4.4 | 600 | Flow 800 gpm at 600 M. No increase for 900 M. |
| | | 1800 | 1.5 | 900 | Flow 5800 gpm at 1500 M. |
| 2 | 3 | 4000 | 0.5 | 2600 | Stream joined by another of 800 gpm flow at 2600 M. |
| | | 4800 | 0.41 | 3500 | No checking done beyond 6100 M. |
| 3 | 2 | 600 | 2.2 | 5740 | Sluice box placed 5740 M. below point of application. |
| | | 7000 | 0.18 | 5000 | Flow 7000 gpm at sluice box. Flow 15000 gpm at 10740 M. |
| 4 | 5 | 25000 | 0.13 | 10000 | 50% kill for an additional 1000 meters. Flow had increased to 40000 at 10 km. mark. |

[1] The first three tests are divided on the basis of increased flow. The total distance of kill from point of application is the sum of the two figures given.

decline in adult population was, however, greater and took place sooner in the treated area than the decline in the untreated area. Two other factors which may have played a role in maintaining the population in the treated area are the possibility of considerable infiltration of adults from adjacent untreated areas, since the maximum flight ranges of the species present is not known, and the possibility that enough very small seepage or spring-fed trickles were overlooked in the treatment to supply fair numbers of adults. Some support for the last supposition is given by the fact that reduction of *Simulium metallicum*, which breeds in fair sized streams, was relatively greater than reduction of *S. ochraceum*, which seems to prefer the smallest seepage streams.

Attention was next turned to the second phase of the investigation, the development of economically practicable methods of application. The use of drip cans was considered impractical, as very large numbers would be needed and it was felt that the cost would be too great and that they were unlikely to be satisfactory dispensers of an emulsion of this nature. The use of absorbent solids appeared to offer considerable promise, as it was felt that cakes or blocks would be a simple way of dispensing the DDT, doing away with the time factor in application, and making it relatively simple to train the cheapest labor to do the actual application. It was hoped also that it would be possible to find some method of prolonging the treatment to such an extent that the breeding streams could be "sterilized" as it were, for periods longer than the length of adult life. This, we believed, might reduce the number of treatments necessary for control to three or four, or possibly fewer per year.

Table 3 lists the various substances tried as absorbents and the results obtained. A number of the blocks gave quite satisfactory kills, but testing was hampered by the fact that one successful trial spoiled a whole stream for further testing for several weeks, and the number of accessible streams was rather limited.

The use of plaster of paris blocks, sponge gourds, or sacks of sawdust with solutions of DDT seem promising methods of application, especially for the smaller streams. Additional trials to find the best and cheapest combination should be initiated.

Since the cost of the xylene-*Triton*-DDT emulsion is relatively high, and all three ingredients must be imported, efforts were made to find substitute solvents and emulsifiers, especially substances obtainable in Guatemala. It was found that at room temperature (about 20° C) 100 cc. of commercial turpentine purchased locally would dissolve about 12 grams of DDT. In a letter from Dr. E. F. Knipling of the U. S. Dept. of Agriculture, dated January 4, 1945, he stated that his group has been able to dissolve about 14 per cent

Table 3

| No. | Form Applied | Composition | Where Applied | Conc.[3] ppm. | Gals. Per Min. | Results |
|---|---|---|---|---|---|---|
| 1. | Cast block | 25 g.pl. of paris<br>25 cc. stock<br>25 cc. water | small stream | 0.44 | 50 | Effective kill. Lasted a little over 24 hours. |
| 2. | Cast block | 25 g. plaster<br>25 g. DDT<br>35 cc. 5% *Triton X-100* in water | sluice box | 0.11 | 200 | 80% kill. Lasted less than 24 hours. |
| 3. | Cast block | 25 g. plaster<br>25 g. DDT<br>50 cc. water | sluice box | 0.11 | 200 | No apparent kill. Lasted a little over 24 hours. |
| 4. | Cast block | 50 g. plaster<br>50 cc. of a mixture of 25 cc. water, 50 cc. turpentine & 10 g. DDT | small stream | [1] | 100 | Effective kill. Lasted 48 hours. |
| 5. | Cast block | 50 g. plaster<br>35 cc. of a mixture of 25 cc. water, 50 cc. turpentine & 6 g. DDT | sluice box | [1] | 200 | 90% kill. Lasted a little over 24 hours. |
| 6. | Cast block | 50 g. plaster<br>35 cc. of a mixture of 17.5 cc. water, 50 cc. turpentine & 6 g. DDT | sluice box | [1] | 200 | Effective kill. Lasted 24 hours. |
| 7. | Cast block | 50 g. plaster<br>30 cc. water<br>10 cc. sol. of 6 g. DDT in 50 cc. turpentine. | small stream | 0.04 | 100 | Effective kill. Lasted 4 days. |
| 8. | Cast block | 50 g. plaster<br>30 cc. water<br>15 cc. sol. of 6 g. DDT in 50 cc. turpentine. | small stream | 0.12 | 50 | Effective kill. Lasted 3 days. |
| 9. | 12 oz. cans filled with various mixtures, plugged with plaster & with nail holes in sides and bottom. | 170 g. sawdust<br>200 cc. stock | stream | 0.18 | 1200 | Effective kill. Exhausted after 6 days when live larvae found on can. |
| 10. | " | 226 g. granulated charcoal<br>100 cc. stock[2] | stream | 0.14 | 800 | Not effective. Plaster plug dissolved after 24 hours. |
| 11. | " | 382 g. coarse salt<br>70 cc. stock[2] | stream | 0.019 | 4000 | 95% kill. Exhausted after 6 days and can half filled with sand. |
| 12. | " | 500 g. plaster<br>135 cc. stock[2] | stream | 0.5 | 300 | No kill after 24 hours and 60 hours. Plaster still intact. |

[1] Unable to calculate concentration because turpentine leaked out during setting of plaster.
[2] Stock referred to consisted of 25 per cent DDT, 7 per cent *Triton X-100*, 68 per cent xylene.
[3] Concentrations in all cases are included only for a basis of comparison and are calculated on a period of diffusion of one hour.

Table 3.—(Continued)

| No. | Form Applied | Composition | Where Applied | Conc.[3] ppm. | Gals. Per Min. | Results |
|---|---|---|---|---|---|---|
| 13. | " | 75 g. sawdust<br>50 g. plaster<br>200 cc. stock[2] | stream | 0.22 | 1000 | Effective kill 24 hours. About half contents dissolved. Can moved upstream 50 yds. No further kill after 5 days when can empty. |
| 14. | Plaster of paris blocks | 29 blocks 3×3×$\frac{3}{4}$" which had absorbed 700 cc. of 4% DDT emulsion. | 5 small streams | 0.19 | 650 | Effective kill for 200 meters below last block in 5 hours. Previews treatment prevented further checking. |
| 15. | "Esparto" sponge gourds set with plaster | Sponge gourd set with plaster and allowed to absorb 350 cc. 4% DDT emulsion. | stream | 0.18 | 350 | Effective kill for 300 meters where stream joined large river. Plaster all washed out in 24 hours. |
| 16. | " | As above, but plaster set with 4% DDT emulsion instead of water. After drying absorbed more emulsion to a total of 650 cc. | large stream | 0.033 | 3500 | Effective kill for 1500 meters. Sponge placed at junction of stream used in test #15 with large stream. Plaster all washed out in 24 hours. |
| 17. | Sawdust in cloth sack. | 0.8 gal. 25% DDT, 7% *Triton*, 68% xylene absorbed by sufficient sawdust to give a moist mix. | river | 0.13 | 25000 | Perfect kill for 6 km. in 48 hours. No kill at 11 kms. Sack moved upstream 50 yds. and checked several days later but no further kill. |

DDT in turpentine at 27°–30° C and up to 25 per cent by warming, which agrees fairly closely with our results. Although xylene will dissolve about 4.5 times as much DDT as turpentine, it cost nearly 7 times as much in Guatemala. It would, therefore be possible to effect a considerable saving in materials cost to use turpentine in place of xylene in making up the concentrate.

The berries of the tree *Sapindus saponaria* are used locally for washing clothes and contain a relatively high proportion of saponin. A crude alcoholic extract of these berries was tried as an emulsifying agent. By its use a good emulsion of turpentine and water was formed which, however, was stable for only a short time, separating into two distinct layers in about an hour. Its homogeneity could be immediately restored by shaking. There is a possibility that crude saponin extracted from these berries could be used as an emulsifying agent, although methods of extraction and costs would have to be worked out, as well as the best proportions to use.

During the course of the tests with turpentine, a single field test was made using turpentine as a solvent, but without an emulsifying agent. Ninety-five grams of DDT were dissolved in 950 cc. of turpentine, and to this was added 2835 cc. of kerosene, to make one gallon. The kerosene was added to make bulk, since the solution had to be poured into a stream during a period of an hour, and the larger volume of liquid could be more evenly poured. A stream with an estimated flow of 4000 gals per minute, and having an adequate larval population, was selected and the kerosene-turpentine-DDT solution poured in throughout a period of 1 hour. This gives a concentration of approximately 1 part DDT to 10 million parts of water. The turpentine

and kerosene could be seen as a thin oily film on the water for some distance downstream. Checking the following day showed but two living larvae over a distance of 2580 meters. These two larvae were at 1500 meters, not far from the junction of a small side stream. No larvae were found in the sluice box 2100 meters from the point of application.

Since neither turpentine nor kerosene is miscible with water and no emulsifying agent was used, we believe that the results observed in this test were due to actual solution of the DDT in water. DDT is said to be soluble in 10 to 15 million parts of water.

To test further the possibility that enough DDT can be dissolved in water to give practical kills, an additional test was run. To 226 grams dry DDT was added 100 cc. *Triton* and the two were mixed to a smooth paste. Water was added gradually to form about a gallon of suspension and the whole was poured gradually, with frequent stirring and shaking, into a river estimated to have a flow of about 8500 gals. per minute. This dosage applied through a period of an hour, gives a concentration of about 1 in 10 million. Larvae of *Simulium* were very scarce immediately below the point of application but at a distance of 2 kilometers downstream were fairly abundant. After treatment no larvae could be found for a distance of approximately 4 kilometers from the point of application. We believe that soap or saponin or any other wetting agent could have equally well been substituted for the *Triton* in this case.

With the demonstration that DDT in the form of very dilute suspensions will give effectual kill the problem of methods of application changes. The search for cheaper and more effective solvents and emulsifiers becomes unnecessary and attention may be directed towards dispensing methods suitable for use by relatively untrained personnel. The following possibilities suggest themselves: (1) a powdered or fine granular combination of DDT and soap; (2) a paste of soft soap and DDT to be mixed with water at the time of application; (3) bars of quickly soluble soap containing DDT to be placed in the smallest streams and seepages.

SUMMARY.—Tests of DDT as a larvicide to control *Simulium* breeding in swift flowing mountain streams in Guatemala are reported.

Using an emulsion containing 4 per cent DDT made from a stock concentrate containing 20 per cent DDT, 20 per cent *Triton X-100* and 60 per cent xylene, complete eradication of *Simulium* larvae from streams for distances up to 10 kilometers was effected at concentrations of 1 part DDT to 10 million parts water.

Efforts to prolong larvicidal action by absorbing emulsions and solutions of DDT on porous substances were unsuccessful. However, solutions of DDT absorbed by porous substances offer possibilities as a method of application for treatment of small streams.

Trials of turpentine and crude saponin from soap berries indicate that they are moderately effective as solvent and emulsifying agent respectively, and might economically replace xylene and *Triton* in Guatemala.

Further tests show that DDT powder made into a suspension in water with a wetting agent gave kills as satisfactory as those obtained with emulsions.[1]—1-10-46.

[1] Printing costs paid by the Gorgas Memorial Laboratory to secure early publication.