THE ACTIVITY OF SULPHONAMIDES AGAINST LARVAE OF *ANOPHELES STEPHENSI* (LISTON), *AEDES AEGYPTI* (L.) AND *LUCILIA SERICATA* (MEIG.) [1]

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ABSTRACT. The insecticidal activity of the sulphonamides, benzene sulphonamide, sulphaquinoxaline and sulphormethoxine has been investigated against larvae of *Anopheles stephensi, Aedes aegypti* and *Lucilia sericata*. The activity of sulphadimethoxine was studied with *L. sericata* larvae. It was found that benzene sulphonamide and sulphaquinoxaline are larvicidal against *An. stephensi* and *Ae. aegypti*. Benzene sulphonamide was found larvicidal against *L. sericata* larvae. All other insecticide tests were negative.

An additional delayed, larvicidal effect was observed in the case of sulphaquinoxaline and larvae of *An. stephensi*, but not with larvae of *Ae. aegypti*. On the other hand, many of the larvae of *An. stephensi* which were apparently moribund after exposure to benzene sulphonamide recovered in the following 24 hours.

No differences between the egg-laying activity of *An. stephensi* and *Ae. aegypti* were observed when larvae and pupae were exposed to high concentrations of benzene sulphonamide, sulphaquinoxaline and sulphormethoxine.

INTRODUCTION

The first investigation of the insecticidal properties of sulphonamides concerned *p*-chloro and *p*-bromobenzene sulphonamides, used against the codling moth, *Curpocapsa pomonella* (L.) by Siegler and Haller (1942). In addition to the direct kill of some developmental stages of an arthropod exposed to a sulphonamide, several investigators found that there was "latent toxicity" viz., the delay of insecticidal activity of a chemical applied to a larva until the insect reaches the adult stage. Because of the general interest in the sub-lethal effects of insecticides on arthropods, and a renewed interest in insecticidal sulphonamides, it was suggested that these compounds should be examined especially with reference to possible latent effects against the larvae of *Anopheles stephensi, Aedes aegypti* and *Lucilia sericata*.

MATERIALS AND METHODS

The insects which were used were as follows:

1. *Anopheles stephensi*. The strain was originally collected near Delhi, India in 1947. Eggs laid in tap water and usually hatched within 24 hours. Newly-hatched larvae were exposed to different concentrations of insecticide, and the mortality rate was calculated (World Health Organization, 1970). Tests were repeated at intervals of 24 hours for about 10 days until larvae reached the pupal stage, and pupae were also exposed to the highest concentrations of the chemicals. For all tests 50 ml beakers were used, into each of which had been pipetted 25 larvae. The mortality rate was observed after 24 hours (WHO, 1963, 1970). Newly-hatched larvae were fed for 2 days with dog biscuit; after this time one yeast tablet was added to each dish on alternate days.

2. *Aedes aegypti*. The reF^{m} strain of *Ae. aegypti* (Macdonald and Sheppard, 1965) was used. Eggs were deposited on filter paper, dried for 2–3 days, and placed in hay infusion for hatching. To have larvae of the same age, filter papers containing eggs were removed from culture trays 2 hours after hatching began. Developing larvae were fed on yeast, and the pupal stage was reached after 6 days. For the tests with sulphonamides, larvae were used from 4 hours after hatching and at 24-hour intervals until they pupated. Pupae were tested only with the highest concentrations of the sulphonamides used for the study.

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3. *Lucilia sericata*. The culture of *Lucilia sericata* was obtained from the Parasitology Department of the Central Veterinary Laboratory (Ministry of Agriculture, Fisheries and Food), New Haw, Weybridge, Surrey. This was maintained in the insectaries of the School of Tropical Medicine in the pupal, adult, oviposition and early larval stages, but as each culture developed to the second larval instar (and began to smell offensively), it was transferred to a ventilated insectary in the Biochemistry Department of the University. The School insectaries had a temperature of about 25±1° C and 75–80% relative humidity, and the Biochemistry insectary a temperature of 22±1° C. Adult flies were fed water and milk (on cotton wool pads in petri dishes) and sugar lumps. As each batch of adults attained sexual maturity, at about 7 days, they were allowed access to bovine liver for oviposition.

Eggs were left on the liver, which was removed from the cage to a shallow dish or wax pot sealed with three layers of fine netting. Larvae hatched in 8–24 hours (usually overnight) and began to feed on the liver. A modification of the technique was employed for the experiments on the exposure of larvae to sulphonamides. Small polystyrene pots of capacity 20 ml. were used, and in each was placed 10 g. of liver mince, to which had been added the appropriate amount of chemical, and 25 1st-stage larvae.

Just before the 3rd instar larvae completed their feed, usually about day 8–10, the rotting liver and larvae, in their container—now opened and placed on its side —were put on sawdust in a glass jar. The larvae moved away from the liver and out into the sawdust, where they pupated. After the larvae had had sufficient time to pupate (day 12), the pupae were counted and the percentage pupation calculated from the known number of original first instar larvae. The percentage yield of adult flies from the pupae was also calculated.

Throughout these studies all observed mortalities were corrected for natural mortality by Abbott's formula (Abbott, 1925).

The sulphonamides used for experiments were sulphaquinoxaline sodium salt (May and Baker Ltd.), sulphormethoxine base (Roche Ltd.) and benzene sulphonamide (British Drug Houses Ltd.). Larvae were exposed to concentrations of the sulphonamide up to maximum solubility. Sulphormethoxine and benzene sulphonamide are practically insoluble in distilled water and so were dissolved in a 0.25% solution of sodium carbonate in distilled water. In the case of *Lucilia sericata* 0.5 ml. of each particular solution was added to 10 g. of minced liver. Small polystyrene pots of capacity 20 ml. were used as containers. Benzene sulphonamide is not soluble in distilled water, requiring a high concentration of sodium carbonate to dissolve it. Since such high concentrations of sodium carbonate may cause mortality, the pure drug was added to 20 g. of minced liver and well mixed; 10 g. of this mixed liver was added to 10 g. of minced liver and well mixed. This serial dilution in liver was repeated until a suitable low concentration was reached.

RESULTS

1. BENZENE SULPHONAMIDE. This compound was used at the rate of 0.0015, 0.0031, 0.0062, 0.0125, 0.05, 0.1 and 0.2%.

An. stephensi. As the larvae grew their tolerance to benzene sulphonamide increased: the LC_{50} for day old 1st stage larvae was about 0.0039% and for 4th instar larvae (9 days old) 0.14%, i.e. about 35 times more than for 1st instar. It was also observed that the newly molted 3rd and 4th instar larvae were more susceptible to the sulphonamide than were late 2nd and late 3rd instar larvae.

When 4th stage larvae were exposed to 0.2% benzene sulphonamide, the "dead and moribund" rate after 24 hours was 75.5%. After 24 hours' recovery in fresh water the mortality rate decreased to 30.4%; in other words, 45.1% recovered from the moribund state.

Ae. aegypti. When *Ae. aegypti* larvae were exposed to benzene sulphonamide it was found that the newly hatched larvae were more tolerant than were day-old lar-

vae, the LC_{50}'s being about 0.18 and 0.006% respectively. As in the case of *An. stephensi,* the tolerance of *Ae. aegypti* then increased as the larvae developed, and late 4th stage larvae were not affected by the highest concentration of 0.2%.

L. sericata. Newly hatched larvae were allowed to feed in the liver and to proceed to the pupal stage. The mortality rate was observed in the pupal stage. This compound was effective against 1st stage larvae of *L. sericata* at 0.2% and the observed mortality rates with 0.2%, 0.4% and 0.8% were 93.4%, 96.6% and 100%, respectively. LC_{50} and LC_{90} were 0.076 and 0.25% respectively. The adults which emerged from these pupae were apparently normal and were not found significantly different from controls in either sex ratio or egg-laying activity.

2. SULPHAQUINOXALINE. This compound was used at the rate of 0.0125, 0.025, 0.05, 0.1, 0.2 and 0.4% in distilled water.

As with benzene sulphonamide, the tolerance of mosquito larvae to sulphaquinoxaline increased with age: for day-old larvae of *An. stephensi* the LC_{50} was 0.0126, while for 4th stage larvae (on day 9) it increased to 0.12%.

On day 6, when 30–42% of the larvae had molted to the 3rd instar in both test solution and control, an increase in susceptibility was observed, and this stage was also more susceptible than late 2nd stage. Newly hatched larvae of *Ae. aegypti* tolerated a higher level of this drug than did day-old larvae, and 0.2% sulphaquinoxaline killed all day-old larvae; the LC_{50} and LC_{90} were about 0.0126 and 0.19% respectively.

When 4th stage larvae of *An. stephensi* were exposed to 0.2% of sulphaquinoxaline the apparent "mortality" rate (dead and moribund) after 24 hours was 26%, but after 24 hours' recovery in fresh water the total mortality rate increased to 66% and eventually only 10% of the larvae pupated.

When 4th instar larvae of *Ae. aegypti* were exposed to 0.2% and 0.4% of sulphaquinoxaline the apparent "mortality" rate after 24 hours' exposure was 89 and 81%. After 24 hours' recovery the final time mortality for 0.2 and 0.4 had fallen to 57.3 and 75.0%.

Against *L. sericata,* sulphaquinoxaline was used in the liver at the rate of 0.05, 0.1, 0.2, 0.4 and 0.8%. At the highest concentration only 10.6% mortality was observed, i.e. similar to the expected mortality in the controls.

3. SULPHORMETHOXINE. Sulphormethoxine was used at dosage levels of 0.0125, 0.025, 0.05, 0.1, 0.2, 0.25, 0.5 and 1.0%. It was dissolved in 0.5% sodium carbonate in distilled water for concentrations up to 0.2%, and in 0.75% sodium carbonate for higher concentrations.

Some of the newly hatched larvae of *An. stephensi* died following exposure to sodium carbonate solutions in excess of 0.5%, and the control mortality was more than 20% with 0.75% sodium carbonate.

The mortality of newly molted 4th stage larvae with 0.0125% to 1.0% sulphormethoxine was 25–86%, and the LC_{50} for this particular stage was about 0.35%. With older 4th stage larvae, the 24-hour mortality sharply decreased, and with 1.0% sulphormethoxine it was only 10–12%.

Pupae exposed to 1.0% were unaffected, and although 10% failed to emerge and 6% died after emerging, the mortality in the controls was almost the same, at 13%. There was no significant difference between the oviposition rates in the adults which developed from treated and control pupae. Due to the lack of further supplies of the sulphonamide at the time, no experiments on *Ae. aegypti* were carried out.

When *L. sericata* larvae were exposed to 0.05, 0.1, 0.2, 0.4 and 0.8% sulphormethoxine, a maximum mortality of only 12% was observed, in the 0.1% sulphormethoxine. Thus sulphormethoxine compound was also not found effective against *L. sericata* larvae.

DISCUSSION

Benzene sulphonamide and sulphaquinoxaline showed larvicidal activity against

all larval stages of *An. stephensi*, while sulphormethoxine tested on 4th stage larvae was effective only on newly-molted 4th instars and showed no effect on mature ones. Changes were observed in the tolerance of the insecticidal materials against the various larval stages, as for instance in the increased susceptibility of *An. stephensi* after the 2nd and 3rd molts, and of *Ae. aegypti* 24 hours after hatching compared with 4 hours after hatching. Larvae of *Ae. aegypti* became very tolerant to benzene sulphonamide by the end of the 1st larval stage. Pupae were unaffected by the insecticides tested at rates up to 1.0%.

Thomas (1965) reported that the DDT-tolerance of *Culex pipiens fatigans* (=*quinquefasciatus*) larvae increased more than 100 times during the development of larvae. Methoxychlor, dieldrin, gamma-BHC, malathion, dicapthon, and allethrin were also much less toxic to 4th instar than to 1st-instar larvae of *C. p. fatigans* (Paulini and De Sousa, 1962). The difference in susceptibility among different instars was less with carbamate insecticides (Mulla, 1961). In tests with DDT on *Aedes dorsalis* larvae, the tolerance of the 4th instar was 100 times more than that of the 1st instar (Yates, 1950).

It has been reported that newly molted 4th instar larvae of *Anopheles quadrimaculatus* were more susceptible to DDT than 3rd instar larvae (Jones, 1957). In tests with *An. stephensi* and *An. atroparvus* larvae using DDT, Garms (1959) observed that after the third molting there was a decreased tolerance.

Burchfield *et al.* (1953) investigated the effect of heptachlor on *Ae. aegypti* and found that the initial tolerance of the newly hatched larvae was very high but dropped rapidly during the first 12 hours. Sulphormethoxine was not effective at the concentration used except for newly molted 4th stage larvae.

Previous studies (Beesley and Peters, 1968, 1971) have shown that sulphaquinoxaline has some systemic activity. Imagicidal effects were observed when this drug was fed to mosquitoes in sugar solution or at dosages which were not toxic to mice.

A delayed-recovery phenomenon was recorded in *An. stephensi*.

When 4th stage larvae were exposed to 0.2 sulphaquinoxaline, after 24 hours' recovery the mortality rate increased from 26% to 66%. The same concentration of benzene sulphonamide caused no such effect and the mortality rate decreased from 75.5% to 30.4%.

After-effects of DDT and dieldrin on *An. stephensi* were reported by Garms (1961), and on *An. atroparvus*, *An. stephensi* and *An. gambiae* by Rehm *et al.* (1958) who mentioned that the mortality rate after exposure to 0.005 ppm of dieldrin increased from 1.7% to 30.4%. Their observed increase was more in the case of *An. stephensi* than *An. atroparvus*, but DDT caused no such effect (Garms, 1961); with DDT many of the moribund larvae recovered. The after-effects of benzene sulphonamide and sulpha-quinoxaline on *An. stephensi* are slightly similar to those of DDT and dieldrin respectively.

During this study it was found that 1st stage larvae of *L. sericata* were susceptible to benzene sulphonamide with LC_{50} and LC_{90} figures of 0.07 and 0.25% respectively. Other sulphonamides tested did not show activity against 1st instar larvae of *L. sericata* at the concentrations used. This is interesting in view of previous studies by Greenwood and Harrison (1965) who showed that 4-methylbenzene-1,3-disulphonamide is very effective against *L. sericata* larvae.

References

Abbott, W. W. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265–267.

Beesley, W. N. and Peters, W. 1968. The chemotherapy of rodent malaria. VI. The action of some sulphonamides alone or with folic reductase inhibitors against malaria vectors and parasites, Part I: Introduction and anti-vector studies. Ann. Trop. Med. Parasitol. 62:288–294.

Beesley, W. N. and Peters, W. 1971. The insecticidal action of some sulphonamides. J. Econ. Entomol. 64:897–899.

Burchfield, H. P., Redder, A. M., Storrs, E. E. and Hilchey, J. D. 1953. Improved methods for rearing larvae of *Aedes aegypti* (L.) for use in insecticide bioassay. Boyce Thompson Institute Contributions 17:317–331.

Garms, V. R. 1959. Die empfindlichkeit Von *Anopheles atroparvus* und *A. stephensi* (Larven und Imagines) gaganuber DDT in abhangigkeit von alter, ernahrung und populationsdichte. Zeit. fur Tropenmed. und Parasitol. 10:426–441.

Garms, V. R. 1961. Beobachtungen Zum Wirkungsver Lauf Von Dieldrin bai *Anopheles*-Larven (*A. stephensi* und *A. atroparvus*). Zeit. Fur. Tropenmed. und Parasitol. 12:262–279.

Greenwood, D. and Harrison, I. R. 1965. The search for a veterinary insecticide. 1–Sulphonamides and Di-sulphonamides active against sheep blowfly. J. Sci. Food Agric. 16:293–299.

Jones, J. C. 1957. A study of possible modifications of the WHO method for testing DDT-resistance in mosquito larvae with special reference to *Anopheles quadrimaculatus* (Say). Bull. Wld. Hlth. Org. 36:353–357.

Macdonald, W. W. and Sheppard, P. M. 1965. Cross-over values in the sex chromosomes of the mosquito *Aedes aegypti* and evidence of the presence of inversion. Ann. Trop. Med. Parasitol. 59:74–87.

Mulla, M. S. 1961. Susceptibility of various larval instars of *Culex p. quinquefasciatus* Say to insecticides. Mosq. News 21:320–323.

Paulini, E. and De Sousa, P. S. 1962. A suscetibilidade do *Culex fatigans* a varios insecticidas na sua evolucao aquatica. Revista brasileira de Malariologia E doencas Tropicais 14:63–78.

Rehm, W. F., Garms, R. and Weyer, F. 1958. Zur nachwirkung von Dieldrin bei Anopheles-Larvan. Zeit. fur Tropenmed. und Parasitol. 9:200–204.

Siegler, E. H. and Haller, H. L. 1942. Toxicity of sulphonamide and related compounds to the codling moth larvae. J. Econ. Entomol. 35:784.

Thomas, V. 1965. Effects of certain extrinsic and intrinsic factors on the susceptibility of larvae of *Culex pipiens fatigans* Wied. to DDT. Mosq. News 25:38–43.

World Health Organization. 1963. Insecticide resistance and vector control. 13th Report of the Expert Committee on Insecticides. Tech. Rep. Series No. 265, 51–55.

World Health Organization. 1970. Insecticide resistance and vector control. 17th Report of the Expert Committee on Insecticides. Tech. Rep. Series No. 443.

Yates, W. W. 1950. Comparative resistance of different instars of *Aedes* mosquitoes to chlorinated hydrocarbon insecticides. J. Econ. Entomol. 43:944–945.