ACCUMULATIVE EFFECTS OF REPEATED ABATE GRANULAR TREATMENTS IN WATER STORAGE DRUMS

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A knowledge of the accumulative and residual characteristics of any insecticide is vital to those concerned with its use in vector control. Such information is important to the effective operational use of the toxicant and it also acts as assurance against the possibility of exceeding acceptable tolerance levels for other organisms. Preliminary field tests in the Virgin Islands (Brooks *et al.,* 1965) and simulated applications (Brooks and Schoof, 1965) as well as studies with a single treatment regime of a granular Abate [3] formulation (Brooks *et al.,* 1966) showed this material to be an excellent mosquito larvicide. To assist further in the evaluation of this compound for field application, studies were designed to establish rates of release of the compound from the granule into a water substrate and the degree of accumulation of the chemical in the water that results from repeated applications.

MATERIALS AND METHODS. Twenty-four 55-gallon drums, each with one end removed, were filled with water at the test site. A 1 percent granular formulation of Abate [4] was selected for application, and treatments were made at dosage levels of 0.1, 1.0, and 2.5 p.p.m. Bentonite and sand granule formulations were used in 6 and 14 drums, respectively. The remaining four drums were left as untreated controls. Biological and chemical analyses were made weekly to assess the toxicant present in the water. Three series of treatment regimes were conducted as follows:

a. Series I: Treatments were made with a 1 percent formulation of Abate on sand in drums containing 50 gallons of water each, at the above-mentioned dosages. This treatment series provided a basis upon which to compare the multiple re-treatment cycles of the sand materials.

b. Series Ia: Eight drums, four containing 17 gallons and four containing 50 gallons of water, with known concentrations of Abate (0.01 to 0.57 p.p.m.) present were periodically re-treated with 18.9 or with 1.89 grams of 1 percent Abate sand granules. Re-treatments were carried out once or twice over a 6-week period.

c. Series II: Abate treatments as sand or bentonite granules were placed in 12 drums, each with 50 gallons of water, at the three dosages indicated to permit comparative analysis of two different formulations. Re-treatments were carried out at the same dosages at 3- and 6-week intervals.

Evaluation of the biological effectiveness of each treatment was made weekly using 3rd instar *Aedes aegypti* larvae of the DDT-dieldrin resistant Charlotte Amalie strain. The criterion of effectiveness was selected at 70 percent kill after 24 hours of exposure or failure to reach the pupal stage. Test specimens were counted into paper containers, transported to the field location, and introduced into the drum as shown in Fig. 1.

Weekly water samples were taken from the upper surface layers of the drum.

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[3] Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or the U. S. Department of Health, Education, and Welfare.

[4] Furnished through the courtesy of the American Cyanamid Company, Princeton, New Jersey.

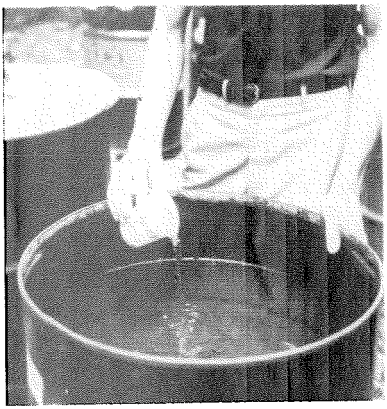

FIG. 1.—Planting third instar *A. aegypti* larvae in Abate-treated 55-gallon water storage drums.

Abate concentrations in the water were determined by extraction of these 450 ml. water samples with CCl_4 and evaporation of the solvent to isolate the insecticide. The residues were then oxidized with perchloric acid and the resulting orthophosphate was determined colorimetrically. Water was analyzed at 48 hours after initial treatment and at weekly intervals thereafter. One pint of the treated water from each dosage and/or test condition, together with a sample from one or more control drums, was brought to the laboratory. Tap water blanks were concurrently analyzed as a check on method and technique.

RESULTS. *Series I:* Data obtained for comparison of a 3- and 6-week treatment cycle with 1 percent sand are seen in Table 1. At the 2.5 p.p.m. dosage, the drums re-treated at 3-week intervals over the test period yielded a recovery of Abate averaging 1.20 p.p.m./week with a high of 1.78 p.p.m. Those drums treated at 6-week intervals averaged 0.78 p.p.m./week with a high of 1.64 p.p.m. recorded. Initial treatment yielded a high of 1.64 p.p.m. after 1 week and declined to 0.20 p.p.m. at the point of re-treatment.

Drums receiving the same initial treatment but re-treated at 6-week intervals averaged 0.31 p.p.m./week; the highest reading, 0.66 p.p.m., occurring at 48 hours after application. Thereafter the concentration dropped to 0.11 p.p.m. in the week prior to the next scheduled treatment.

Dosages of 0.1 p.p.m. Abate applied in drums at 3-week intervals averaged 0.05 p.p.m./week with a high of 0.07 p.p.m. Those treated every 6 weeks averaged 0.03 p.p.m./week with a high of 0.08 p.p.m. The 0.04 p.p.m. seen at 48 hours with this dosage level declined to 0.02 p.p.m. in the week preceding the second treatment.

Series Ia: Results of the tests on concentration accumulation are presented in Table 2. The 50 gallons of water re-treated at 3-week intervals with the 18.9 g. of 1 percent Abate granules had an average of 0.49 p.p.m. of toxicant with a high of 0.79 p.p.m., as compared with the 18.9 g. treatment of 17 gallons which had an average of 0.77 p.p.m. and a high of 1.72 p.p.m. The 50 gallons of water re-treated at 6-week intervals with the 18.9 g. dosage showed an average of 0.27 p.p.m. of toxicant per week and a high of 0.53 p.p.m., as compared to the treatment in 17 gallons, which averaged 0.44 p.p.m. and reached a high of 1.23 p.p.m.

Drum re-treatments of 1.89 g. of 1 percent Abate sand granules in 50 gallons at 3-week intervals had an average of 0.05 p.p.m. of toxicant with a high of 0.08 p.p.m., while the same amount in 17 gallons produced an average of 0.07 p.p.m. of Abate and a high of 0.10 p.p.m. The average p.p.m. values recovered from the 1.89 g. treatment on a 6-week regime was 0.03 p.p.m. and 0.06 p.p.m. in 50 and 17 gallon treatments, respectively. High values for the 50-gallon treatment were 0.06 p.p.m. against 0.14 p.p.m. in 17 gallons.

Series II: As shown in Table 3, drums treated with a 2.5 p.p.m. dosage at 3-week intervals with 1 percent Abate sand averaged 1.20 p.p.m. of toxicant per week, while Abate bentonite granules averaged 0.22 p.p.m./week. The highest toxicant level recorded for the sand granules was 1.78 p.p.m. and the high for the bentonite formulation, 0.38 p.p.m. The same

TABLE 1.—Comparison of weekly average concentrations of Abate from single and multiple 1 percent sand granule treatments (3- and 6-week intervals), Savannah, Georgia, 1965.

Treatment p.p.m. dosage	Series	48 hrs. p.p.m.	Weeks average concentration in p.p.m.													Weekly drum average
			1	2	3	4	5	6	7	8	9	10	11	12	13	
2.5	3 week	1.13	1.78	1.10	*1.10	1.08	0.44	*0.77	1.27	1.33	*1.40	1.57	1.47	*1.21	1.23	1.20
	6 week	1.20	1.64	1.07	0.70	0.41	0.25	*0.20	1.16	1.05	0.85	0.71	0.53	*0.38	1.15	0.78
1.0	3 week**	0.58	0.43	0.27	*0.76	0.49	0.35	*0.38	0.55	0.36	*0.66	0.48	0.53	*0.76	0.71	0.53
	6 week**	0.66	0.51	0.33	0.18	0.15	0.11	*0.17	0.54	0.26	0.24	0.16	0.22	*0.60	0.46	0.31
0.1	3 week**	0.04	0.03	0.02	*0.05	0.06	0.03	*0.05	0.06	0.04	*0.07	0.07	0.07	.....	....	0.05
	6 week**	0.04	0.03	0.02	0.02	0.01	0.02	*0.08	0.05	0.04	0.03	0.03	0.02	.....	....	0.03

* Weeks of re-treatment.
** Average values of Abate concentration from two replicate drums.

TABLE 2.—Effect of repeated sand granule treatments of Abate on concentrations of toxicant in 50 and 17 gallons of water, Savannah, Georgia, 1965.

Treatment interval	No. gal.	Applied dosage p.p.m./drum	Parts per million recovery each week										Weekly average
			1	2	3[a]	4[c]	5	6[a]	7	8	9[a]	10	
3-week	50	1.0[b]	0.50	0.37	0.79	0.53	0.41[e]	0.53	0.44	0.27	0.74	0.41	0.49
		0.1[b]	0.04	0.03	0.08	0.07[d]	0.03[f]	0.06	0.05	0.04	0.08	0.06	0.05
	17	1.0	....	....	....	....	0.47[e]	1.72	0.57	0.37	0.90	0.56	0.77
		0.1	....	....	....	....	0.05[f]	0.10	0.08	0.04	0.10	0.08	0.07
6-week	50	1.0[b]	0.53	0.38	0.34	0.18	0.14[e]	0.25	0.43	0.21	0.15	0.08	0.27
		0.1[b]	0.04	0.03	0.02	0.01	0.03[f]	0.06	0.04	0.03	0.03	0.02	0.03
	17	1.0	....	....	....	....	0.27[e]	1.23	0.66	0.20	0.17	0.12	0.44
		0.1	....	....	....	....	0.03[f]	0.14	0.06	0.05	0.04	0.04	0.06

[a] Week of re-treatment.
[b] Weeks 1 to 4 average of two drums.
[c] Water volume reduced to 17 gallons in 50 percent of drums.
[d] Based on one drum only (one sample lost in analysis).
[e] Each drum received 18.9 g. of 1 percent Abate-sand granules per treatment.
[f] Each drum received 1.89 g. of 1 percent Abate-sand granules per treatment.

TABLE 3.—Comparison of accumulated Abate concentrations from bentonite and sand formulations, Savannah, Georgia, 1965.

Treatment cycles	Treatment dosage p.p.m.	% Abate per formulation	Week after treatment													
			1	2	3*	4	5	6*	7	8	9*	10	11	12*	13	Avg.
3 Weeks	2.5	1% Sand	1.78	1.10	1.10	1.08	0.44	0.77	1.27	1.33	1.40	1.57	1.47	1.21	1.23	1.20
		1% Bentonite	0.18	0.09	0.18	0.15	.12	.22	0.26	0.15	....	0.33	0.36	0.26	0.38	0.22
	1.0	1% Sand	.49	.31	.79	.52	.35	.38	.55	.35	.66	.48	.53	.76	.71	0.51
		1% Bentonite	.06	.05	.12	.11	.06	.08	.13	.07	.11	.15	.17	.15	.21	0.10
	0.1	1% Sand	.03	.02	.05	.06	.03	.05	.06	.04	.07	.07	.07	....	....	0.04
		1% Bentonite	.01	.01	.01	.01	.01	.02	.02	.02	.03	.02	.02	....	....	0.02
								*						*		
6 Weeks	2.5	1% Sand	1.64	1.07	0.70	0.41	0.25	0.20	1.16	1.04	0.85	0.71	0.53	0.38	1.15	0.78
		1% Bentonite	0.18	0.10	.11	.12	.10	.10	0.21	0.13	.14	.22	.17	.21	0.30	0.16
	1.0	1% Sand	.54	.36	.24	.16	.11	.16	.54	.26	.24	.16	.22	.59	.46	0.33
		1% Bentonite	.06	.06	.06	.06	.05	.05	.08	.09	.07	.09	.09	.06	.10	0.08
	0.1	1% Sand	.03	.02	.02	.01	.02	.08	.05	.04	.03	.03	.02	....	....	0.02
		1% Bentonite	<.01	<.01	<.01	<.01	<.01	<.01	.01	<.01	.02	<.01	<.01	....	....	<0.01

* Weeks of re-treatment.

dosage applied at 6-week intervals yielded an average of 0.78 p.p.m. of Abate per week for sand granules and 0.16 p.p.m. of Abate per week for bentonite formulation. The high recorded for sand was 1.64 p.p.m., the high for bentonite, 0.30 p.p.m.

Drums treated with a 1.0 p.p.m. dosage at 3-week intervals with Abate-sand granules average 0.51 p.p.m. of toxicant with a high of 0.79 p.p.m., while those treated with Abate-bentonite granules averaged 0.10 p.p.m. of Abate with a high of 0.21 p.p.m. At 6-week intervals, the drums with the 1.0 p.p.m. dosage from sand granules averaged 0.33 p.p.m. of Abate with a high of 0.59 p.p.m., while those treated with bentonite granules averaged 0.08 p.p.m. with a high of 0.10 p.p.m.

Drums treated with a 0.1 p.p.m. dosage at 3-week intervals with Abate-sand granules averaged 0.04 p.p.m. of toxicant with a high of 0.07 p.p.m., while those containing the same dosage from bentonite granules averaged 0.02 p.p.m. of toxicant with a high of 0.03 p.p.m. The drums with the 0.1 p.p.m. dosage on a 6-week re-treatment schedule with sand granules averaged 0.02 p.p.m. of toxicant per week with a high of 0.08 p.p.m., while those treated with bentonite granules had an average of <0.01 p.p.m. and a high of 0.02 p.p.m.

DISCUSSION. As expected in Series I, the drums receiving a 3-week treatment had higher average concentrations per week than those receiving the 6-week regime. At all applied dosages the concentration rose after each re-treatment, in some instances rather sharply, in others only slightly. With the most frequent re-treatment (every 3 weeks) the average concentration is at the same general level shown 48 hours after the initial application. Thus, under this treatment cycle at a 1 p.p.m. dosage level, the initial Abate level of 0.58 p.p.m. reflects closely the average of 0.53 p.p.m. The level did not go above 0.76 p.p.m. through the test period. The same general picture appears with the 2.5 p.p.m. treatment with the exception of the higher values and unexplained lower values found in weeks 5 and 6. Significant accumulative increase of Abate in the water did not occur with the Abate-sand granule formulation following continued re-treatment at either 3- or 6-week intervals.

The average concentrations over the test period for the 2.5, 1.0, and 0.1 p.p.m. dosages (Table 1) re-treated at 3-week intervals were approximately one-half the initial applied dosage. With the 2.5 p.p.m. treatment, an average of 1.20 p.p.m. was recovered, with the 1 p.p.m., 0.53 p.p.m., and with 0.1 p.p.m., 0.05 p.p.m. In drums re-treated at 6-week intervals, the average concentration approximated a figure equal to one-third the initial concentration applied (0.78 p.p.m. average for 2.5 p.p.m. treatment, 0.31 p.p.m. for the 1.0 p.p.m. treatment, and 0.03 p.p.m. for the 0.1 p.p.m. treatment). This relationship between re-treatment interval and the percent of toxicant released must be considered in the planning of field applications. The proposed dosage levels will necessarily be correlated with the treatment cycle to insure a continuous biologically effective concentration in the container.

In Series Ia, the drums with the 17-gallon volumes recorded relatively higher levels of Abate after each application than did those containing 50 gallons. Concentration declined as the treatment aged at either volume. The difference observed between the concentrations achieved in the two water volumes also decreased. Each drum, regardless of water volume, was treated with the same amount of granules to simulate field practice, so the higher reading for the 17-gallon drum upon re-treatment was expected. Thus, the dosage applied in the 17 gallons of water was three times greater than that applied in the 50-gallon volume. The actual application rates per water volume for the drums with 17 gallons of water were 3.0 and 0.3 p.p.m.

The reason for treating all drums with a standard amount rather than according to water volume (i.e., all treatments were

applied to achieve a 2.5, 1.0 and 0.1 p.p.m. dosage in 50 gallons regardless of the actual water level in the drum) was that under field conditions a standard dosage yields economical utilization of manpower as well as assurance of maintaining a biologically effective dosage regardless of fluctuations in the water volume. With only two exceptions, concentration of Abate in both water volumes remained at a level less than the initial applied dosage.

In the Series II tests, a significant accumulative increase of Abate in the water did not appear to occur with re-treatments at either 3- or 6-week intervals. Again, the average concentration of Abate in water treated with 1 percent Abate-sand granules at 2.5 and 1 p.p.m. dosages appeared to have a direct relationship to the concentration appearing in drums treated at these same levels with 1 percent Abate-bentonite granules. In the 0.1 p.p.m. treatment, drums treated with sand granules averaged twice the amount of Abate as those treated with bentonite granules. Since newly formulated materials were used in this test, it must be assumed that the bentonite granule binds the Abate more securely than the sand granule; hence a smaller quantity is released from the bentonite granule.

In all the drums in Series I and II, total biological mortalities were obtained throughout the 13-week test period. Drums of the comparative water volume tests in Series Ia gave 100 percent larval mortalities through the 13 and 6 weeks of their respective test periods (Table 4).

SUMMARY. The effect of repeated treatments of Abate at 3- and 6-week intervals upon the concentration of the toxicant in water held in 55-gallon drums out-of-doors was evaluated chemically and biologically at Savannah, Georgia. One percent Abate on sand or bentonite granules was used to treat water at applied concentrations of 0.1, 1.0, and 2.5 p.p.m. Except for one set of drums with 17 gallons, all drums contained 50 gallons of water each. Chemical analyses based on a colorimetric method of phosphate determination were made at 48 hours after initial treatments and at weekly intervals thereafter. Biological assessment was made by weekly exposure of 3rd instar *A. aegypti* (Charlotte Amalie strain) larvae. Findings for sand granule treatments applied at 1.0 and 2.5 p.p.m. of Abate showed that approximately 50 percent of the toxicant is released within 48 hours with the concentration of Abate then gradually declining thereafter. With each re-treatment the concentration rises

TABLE 4.—Weeks of complete mortality of 3rd instar *A. aegypti* larvae with Abate granules, Savannah, Georgia, 1965.*

Series	Treatment regime	Total weeks of testing	Drums per test**	Dosage levels in p.p.m.		
				2.5	1.0	0.1
Series I and II	3-week interval treatment	13	6	13	13	13
	6-week interval treatment	13	6	13	13	13
Series Ia	Comparative water volume treatment	6	4	..	6	6
		6	4***	..	6	6

* All treatments shown at 6 and 13 weeks were 100 percent biologically active at the time of discontinuance of testing.

** Results include all formulations tested.

*** Treatments were made in 17 gallons of water against 50 gallons for all other drums.

rather sharply, but even with the most frequent re-treatment (every 3 weeks) the average level remained approximately the same as that shown at 48 hours after initial application. At the applied dosage of 1.0 p.p.m. of Abate the level of toxicant did not rise above 0.79 p.p.m. during the 13-week period. The same general picture is apparent with the 2.5 p.p.m. treatment. A sustained accumulation of the Abate did not occur with re-treatment at either 3- or 6-week intervals.

To simulate field practices, drums containing 17 gallons of water were treated with the same quantity of granules necessary to provide a 1.0 p.p.m. application in 50 gallons of water. As anticipated, Abate concentrations in drums containing 17 gallons of water remained at a higher level after each re-treatment than those in 50 gallons. The difference in Abate recovery from the two volumes of water decreased with age of treatment but again no significant accumulative effects were noted.

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References

BROOKS, G. D., and SCHOOF, H. F. 1965. Simulated field tests of new insecticide formulations against *Aedes aegypti* larvae in 55-gallon water storage containers. Proc. 52nd Ann. Meet. N. J. Mosq. Ext. Assoc., pp. 160–166.

BROOKS, G. D., SCHOOF, H. F., and SMITH, E. A. 1965. Effectiveness of various insecticides against *Aedes aegypti* infestations in water storage drums in U. S. Virgin Island. Mosq. News 25(4):423–427.

BROOKS, G. D., SCHOOF, H. F., and SMITH, E. A. 1966. Evaluation of five formulations of Abate against *Aedes aegypti*, Savannah, Georgia, 1965. Mosq. News 26(4):580–582.

ANOPHELES STEPHENSI LISTON IN EGYPT, UAR.

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Anopheles stephensi is one of the malaria vectors of the Oriental region. It was first described by Liston (1901), from India. It is considered an efficient vector responsible for city malaria in Bombay, Bangalore and Lucknow, and an important vector under rural conditions in western and northwestern India. In some experimental infections in the laboratory in India (Roy 1943), the percentages of mosquitos acquiring sporozoites were 45.4 for *Plasmodium vivax* and 37.3 for *Plasmodium falciparum*. Infection in nature ranged from 0.2 to 2.6 percent for sporozoites (Boyd 1949).

Within the Oriental Region the distribution of *A. stephensi* lies in India, Pakistan, Burma, and China and, outside this region, it was recorded in the Baluchistan, Iran, Iraq and the Eastern region of Saudi Arabia near the Persian Gulf.

Another variety or subspecies of this species was described by Sweet and Rao (1937) in Marikanave, India, under the name *A. stephensi mysorensis*. Although the two forms are apparently indistinguishable in the larval and the adult stages, Afridi *et al.* (1958) were able to distinguish the subspecies *mysorensis* from typical *stephensi* by means of egg measurements. *A. mysorensis* is considered less hardy than the type form and has more