Journal of the American Mosquito Control Association, 20(4):444–448, 2004

THE USE OF HOUSEHOLD BLEACH TO CONTROL *AEDES AEGYPTI*

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ABSTRACT. We evaluated the lethal effects of household bleach (5.25% sodium hypochlorite; NaOCl) on immature *Aedes aegypti* in tap water, with and without food, and in field-collected automobile tires. A sublethal dose was employed as a disinfectant in tires to control immatures through the destruction of microorganisms that constitute the main food items of mosquito larvae. The concentration of bleach that was required to kill all immatures was higher in the presence of larval food and older immatures. Lethal (100%) concentrations in the presence of food were 16 ppm for 1st instars, 64 ppm for 2nd instars, and 250 ppm for 3rd and 4th instars. A single treatment with 250 ppm of bleach per tire (2 tablespoons per 5 liters of water) killed the larvae, but pupae started to appear 12–17 days later. Total pupal production in 2 months decreased from 118 ± 26 pupae/tire (mean ± SE) in the controls without bleach to 66 ± 5 pupae/tire in treated tires. A single treatment with 250 ppm followed by weekly applications of sublethal doses (50 ppm; a teaspoon) significantly reduced pupal production (2 ± 1 pupae/tire in 2 months). We recommend that whenever a container that produces mosquitoes cannot be eliminated, it would be better to clean it before applying bleach. The combined action of cleaning and bleach is expected to reduce available larval food, reduce the amount of NaOCl for treating the container, and make it less attractive for future mosquito oviposition.

KEY WORDS Bleach, sodium hypochlorite, *Aedes aegypti*, dengue, control, larvicide

INTRODUCTION

Sodium hypochlorite (NaOCl, 5.25%), the active ingredient in household bleach, has been used for over 100 years as a disinfectant and sanitizer because of its high effectiveness and wide spectrum of action against bacteria, viruses, fungi, and algae (Rutala and Weber 1997). Macfie (1915) investigated the properties of chlorine solutions, ranging from 1:500,000 (2 ppm) to 1:10,000 (100 ppm), on *Aedes aegypti* mortality. The tests were conducted in both pure water and water from a larval habitat because chlorine reacts with organic matter, losing some efficacy in killing some living organisms. He found that the 100-ppm dosage killed *Ae. aegypti* larvae in 24 h in both types of water. However, the author concluded that "chlorine would not appear to have any special application as a larvicide" (Macfie 1915, p 71) possibly because large quantities of the product would have to be applied to large water storage containers (e.g., 400 ml of bleach [100 ppm] would be needed in a drum with 200 liters of clean water to kill the mosquito larvae). More recently, household bleach was successfully mixed with household detergent and applied to the inner surface of water storage containers to kill *Ae. aegypti* eggs in a community participation research project in Honduras (Fernandez et al. 1998, Sherman et al. 1998). They also investigated the lethal doses required to achieve 50% mortality in larvae, pupae, and eggs. The range of concentration used in the larval and pupal experiments was 100–2,600 ppm in clean water. Mortality (50%) of larvae was achieved between 27 min and 4 h, and between 4.3 h and 15 h for the pupae. Eggs treated with 100 ppm did not hatch. Ritchie (2001) reported the use of 1% and 5% NaOCl to kill 100% of *Ae. aegypti* larvae in less than 24 h as part of a program to eliminate introductions of this species into Australia on dry cargo or the aquatic stages in imported containers. Bleach concentrations used in those experiments were rather high (10,000–50,000 ppm).

This paper explores the effect of household bleach on each aquatic stage of *Ae. aegypti* to determine lethal doses under adequate feeding conditions and in tap water. It also introduces the notion of using sublethal doses of bleach to control immatures indirectly, that is, through the destruction of microorganisms that constitute the main food items of mosquito larvae (Barber 1928, Merritt et al. 1992).

MATERIALS AND METHODS

We studied the effect of chlorine bleach (5.25% NaOCl; Clorox®) on the mortality of immature stages of *Ae. aegypti* in a series of 3 experiments in which various concentrations of bleach were applied to 1) larvae in dechlorinated tap water and observed for mortality for up to 48 h; 2) larvae in dechlorinated tap water with an optimal diet and observed until adult emergence or death, and 3) larvae in tires that were brought from the field, placed outdoors, and monitored for 2 months. Specimens used in the 1st 2 indoor laboratory experiments came from the 1st cohort of an urban strain collected as eggs in the field (San Juan, Puerto Rico). Mean and SD of temperature and relative humidity in the laboratory during the experiments were 25.1 ± 0.6°C and 78.4 ± 3.0%, respectively (weather station 433 mHz, Oregon Scientific, Cannon Beach, OR).

In the 1st experiment, 50 larvae (3 replicates) were placed in pans containing 300 ml of dechlorinated tap water and 1 of the following bleach concentrations: 0, 2, 4, 6, 8, or 10 ppm. Each larval instar (I–IV) was evaluated separately. Specimens were reared with a diet of liver powder (1 mg per larva per day) until they reached the appropriate stage of development. Larvae were rinsed with tap water to eliminate traces of food and transferred to the experimental pans. After 24 h of exposure, the survivors were counted and transferred to pans with untreated water and observed for an additional 24 h to record their survival. Two 2-way ANOVA were used to test the null hypothesis of lack of significant effects of bleach concentration, instar, and their interaction on mean survival after 24 and 48 h following exposure (arcsine transformed; Statistica for Windows, StatSoft Inc., Tulsa, OK).

Because NaOCl reacts with the organic matter in the water, we expected that the concentration required to kill immature mosquitoes in containers with larval food was going to be higher than in pure water. Thus, in the 2nd experiment, we explored the effect of a single application of NaOCl on each instar under an optimal diet until 100% mortality occurred. Fifty specimens (3 replicates) were placed in plastic pans with 300 ml of dechlorinated tap water and 1 mg of liver powder per larva per day until the specimens reached the desired developmental stage (I–IV, pupa). Each *Ae. aegypti* instar was exposed to a single application of each of the following bleach concentrations and given their daily food supply until adult emergence or death (1st instars = 0, 8, 16, 32, or 64 ppm; 2nd instars = 0, 8, 16, 32, 64, or 128 ppm; 3rd instars = 0, 16, 32, 64, 128, or 256 ppm; 4th instars = 0, 64, 128, or 256 ppm; pupae = 0, 8, 32, 64, 128, or 256 ppm). The range in NaOCl concentrations used in these experiments was wider for the advanced instars so that we would be able to observe 100% mortality in each replicate. We did not use the same concentrations for each instar because we knew that older instars would require higher concentrations. We recorded the number of adults emerging from each experiment. A 1-way ANOVA was used to test the null hypothesis of lack of significant effects of bleach concentration on the mean survival (arcsine transformed) of *Ae. aegypti* for each instar ($\alpha = 0.05$). When a treatment produced 0 survival (i.e., 100 percent mortality) in all the replicates (0 variance), that treatment was not included in the ANOVA.

In the 3rd experiment, we tested for the effects of the 250 ppm bleach concentration (the concentration that killed 100% of 4th instars in the 2nd experiment) on *Ae. aegypti* pupal productivity in automobile tires collected from the field. The 1st 10 tires found with mosquito larvae in San Juan, Puerto Rico, were collected, and their contents of immature *Ae. aegypti* (70 I–II instars and 40 III–IV instars per tire), solids (plant leaves, twigs, fruits, sediment), and filtered (0.1 mm) water (5 liters) were evenly added to each of 9 tires. Three of the tires were left as controls without bleach, another 3 tires received a single application of 250 ppm of bleach at the beginning of the experiment, and the other 3 tires received a single application of 250 ppm of bleach at the beginning of the experiment plus a weekly application of 50 ppm of bleach. The tires were randomly placed flat in a row next to each other outdoors in partial shade and directly exposed to rainfall and natural mosquito colonization. Mean and SD of daily temperature, rainfall, and relative humidity during the experiments (November 2003–January 2004) were 25.3 ± 0.1°C, 6.6 ± 1.5 mm, and 79.7 ± 1.0%, respectively (weather station 433 mHz). Water volume in the experimental tires decreased markedly during the 2nd week of the study, and rainwater was added to each tire to the initial levels. The laboratory in San Juan had a residential area with houses on 1 side and a secondary, urban forest on another side. The 2 other sides were open fields with grassy lawns. Pupae appearing every 2 days were removed and counted, and the experiments were continued for 2 months. Total number of pupae produced per tire in each of the 3 treatments was recorded. A 1-way ANOVA was used to test the null hypothesis of lack of effects of treatments on total pupal production (log transformed) per tire ($\alpha = 0.05$). At the end of the observation period, all living specimens that remained in each of the tires were counted and identified by instar and species.

RESULTS

Experiment 1: Mean survival of *Ae. aegypti* larvae 24 h after initial exposure to bleach in tap water changed significantly (2-way ANOVA) according to concentration ($F = 54.7$, df 5,47; $P < 0.001$) and instar ($F = 246.6$, df 3,47; $P < 0.001$). We found a significant statistical interaction ($F = 11.9$, df 15,47; $P < 0.001$) of bleach concentration and instar on survival (Fig. 1), reflecting the relatively high susceptibility of 1st instars as opposed to 4th instars. Overall survival of 4th (98.7%) and 2nd (91.1%) instars was high, with lower values for 3rd (78.4%) and 1st (20.1%) instars. Overall survival for an additional 24 h after exposure did not change much in 4th instars (95.2%) compared with 3rd (66.8%), 2nd (45.3%), and 1st instars (15.5%). Results of the 2-way ANOVA on mean survival 48 h after initial exposure to bleach showed significant effects according to bleach concentration ($F = 52.7$, df 5,47; $P < 0.001$) and instar ($F = 140.1$, df 3,47; $P < 0.001$). The test showed a significant statistical interaction ($F = 9.1$, df 15,47; $P < 0.001$) of bleach concentration and larval instar on survival. The interaction resulted from the uniformly high survival of 4th instars compared with 100% mortality in 1st instars in all concentrations of bleach that were tested (Fig. 1).

Experiment 2: In general, mean survival of *Ae. aegypti* at each immature stage reared with liver powder in the laboratory significantly varied with bleach concentration (Table 1), but for each instar,

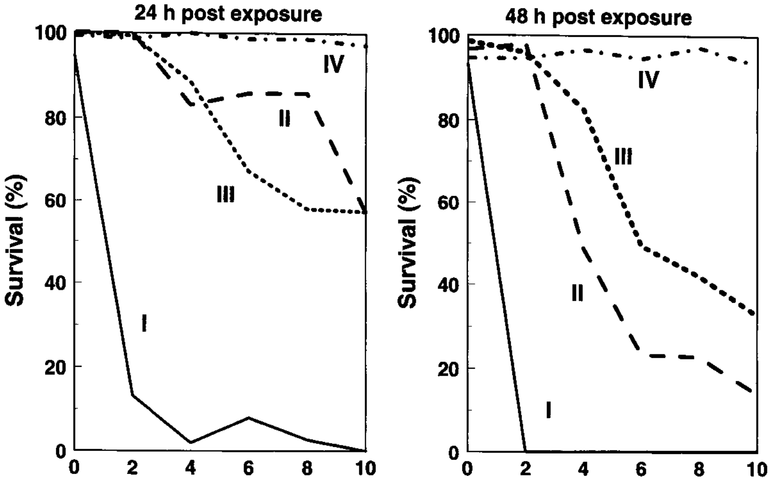

Fig. 1. Survival (%) of larval instars of *Aedes aegypti* 24 and 48 h after initial exposure to a single, initial application of sodium hypochlorite (household bleach) in tap water (0–10 ppm).

there was a concentration that killed all larvae in the experiments (Fig. 2). Bleach concentrations of 16 ppm and higher produced 0 survival on the 1st instars, and for that reason, only the concentrations of 0 and 8 ppm were included in the statistical analysis (Table 1). Pupae were more resistant and the highest concentration used in the experiments (250 ppm) allowed for 33% adult emergence from the initial pupae. Thus, lethal (100%) concentrations in the presence of food were 16 ppm for 1st instars, 64 ppm for 2nd instars, and 250 ppm for 3rd and 4th instars.

Experiment 3: The total production of *Ae. aegypti* pupae (log transformed) during the 2 months of observations in tires changed significantly among treatments (1-way ANOVA; $F = 39.01$, df 2,6; $P < 0.001$). Tires without bleach produced 118 ± 26 pupae (mean ± SE), and their productivity decreased over time (Fig. 3). The number of larvae remaining at the end of the experiments was relatively large (I–II instars = 130 ± 52, III = 67 ± 31, IV = 25 ± 9). Tires with a single application of 250 ppm at the beginning of the experiments began producing the 1st pupae 12–17 days later, with a total 66 ± 5 pupae per tire, and their productivity also declined with time (Fig. 3). Larvae present at the end of the experiment were not as abundant as in the untreated controls (I–II instars = 19 ± 8, III = 47 ± 18, IV = 11 ± 11). Tires with an initial application of 250 ppm and a weekly dose of 50 ppm produced a total of 2 ± 1 pupae per tire in 2 months, and the number of larvae remaining at the end of the experiments was also the lowest observed (I–II instars = 2 ± 1, III = 0.3 ± 0.3, IV = 1.3 ± 1.3). No correlation was found between numbers of pupae produced every 2 days and temperature, rainfall, or relative humidity—only between rainfall and relative humidity ($r = 0.49$; $n = 30$; $P < 0.01$).

DISCUSSION

Results showed that the lethal concentration of bleach was highly variable, depending on the presence of organic matter and immature stages. Because all 1st instar larvae died in the experiments with clean water and 2 ppm of bleach, it appears that chlorinated tap water (allowed maximum residual disinfectant level = 4 ppm, U.S. EPA 1998) will be sufficient to prevent *Ae. aegypti* production in water storage containers. For example, the amount of bleach required for a 2 ppm concentra-

Table 1. One-way ANOVA results of *Aedes aegypti* immature survival to various concentrations of NaOCl under an optimal diet.

		Effect		Error			
Instar[1]	Bleach concentrations (ppm)	df	MS	df	MS	F	P
1st	0, 8	1	0.347	4	0.093	3.70	>0.050
2nd	0, 8, 16, 32	3	0.607	8	0.012	47.60	<0.001
3rd	0, 16, 32, 64, 128	4	0.738	10	0.009	81.44	<0.001
4th	0, 64, 128	2	1.459	5	0.019	75.33	<0.001
Pupae	0, 8, 32, 64, 128, 256	5	0.536	11	0.030	17.48	<0.001

[1] Results from concentrations in which all immautres died in each replicate were not included in the analysis.

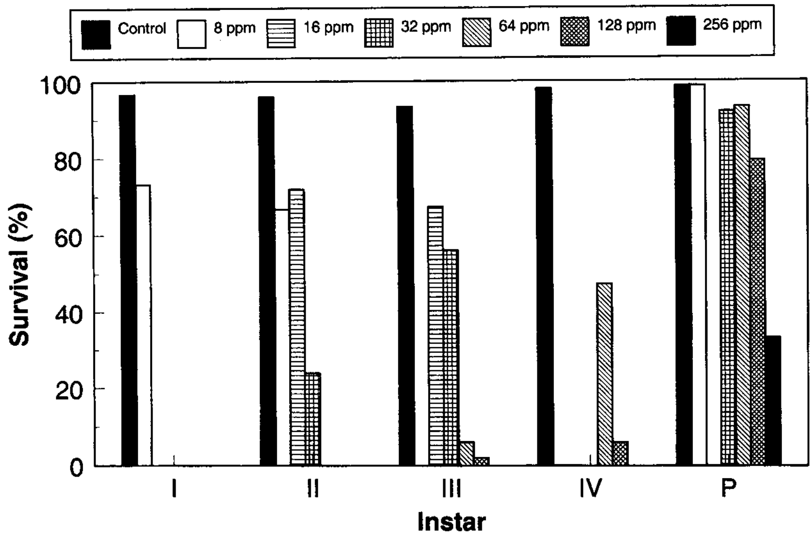

Fig. 2. Survival to adulthood of immature *Aedes aegypti* exposed to a single application of sodium hypochlorite (household bleach) in rearing pans with water and larval food (1 mg/larva/day). The range in NaOCl concentrations tested was not the same for each instar, but wider for the more advanced instars (1st instars = 0, 8, 16, 32 ppm; 2nd instars = 0, 8, 16, 32, 64, 128 ppm; 3rd instars = 0, 16, 32, 64, 128, 256 ppm; 4th instars = 0, 64, 128, 256 ppm; pupae = 0, 16, 32, 64, 128, 256 ppm).

tion in a drum with 200 liters of clear water would be only 8 ml (as a reference, a tablespoon has 14.78 ml). Such a concentration of NaOCl should render the water safe to drink (Rutala and Weber 1997), but if advanced instars are already present, no immediate effect on immature survival will be observed. Given that most water storage containers accumulate a layer of sediment with organic matter at the bottom, it is possible that greater (allowed) concentrations of chlorine would be needed to disinfect the water but would have virtually no effect on *Ae. aegypti* larvae.

The concentrations of bleach required to kill all larval stages in the presence of an optimal diet was higher than in previous experiments in which organic matter was present in the water (Macfie 1915, Sherman et al. 1998). It is likely that part of the free chlorine reacted with the organic matter provided to the larvae as an external source of particulate food, thus leaving a lower effective concen-

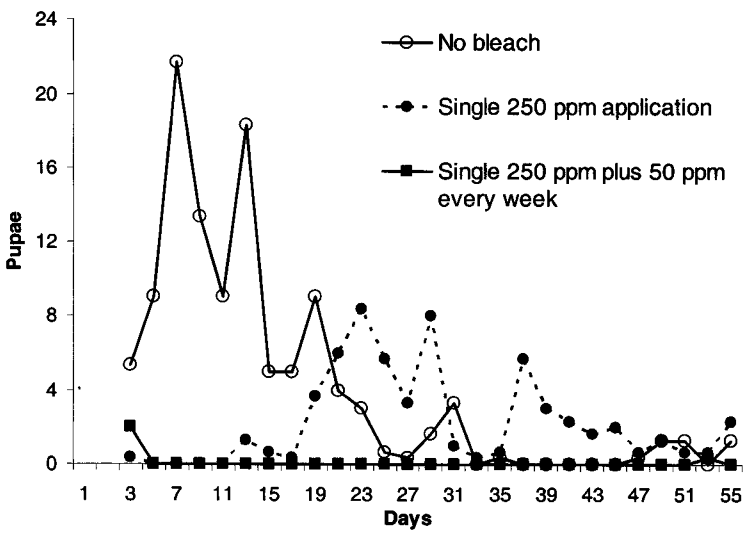

Fig. 3. Pupae produced every 2 days in experimental automobile tires initially collected from the field and exposed to 3 treatments: no bleach, a single application of 250 ppm of bleach, and a single application of 250 ppm plus a weekly application of 50 ppm of bleach.

tration of bleach to influence mosquito immatures. We used an optimal diet to try to establish the dose of NaOCl that would be required to kill all larvae in productive containers. In natural containers, bleach could affect the larvae directly as well as their source of food (organic matter and the microbial community of decomposers and microscopic algae in sun-exposed containers).

The concentration (250 ppm) chosen to test the effect of bleach on larvae in tires was effective as a shock treatment by killing all larvae present at the time of application. The results showed that its effect on mosquito production did not last long because pupae were observed after 12–17 days post-application (Fig. 3). The weekly treatment with a sublethal dose (50 ppm) was sufficient to prevent significant pupal production. This amount is equivalent to 1 teaspoon (5 ml) of NaOCl per tire per week. According to the results (Fig. 2), this concentration should have eliminated any 1st and 2nd instars present at the time of application. The persistence of larvae in those tires that appeared to be in 3rd instar, but without progressing any further, might indicate that they were either impaired or starving and could not molt. Because some 3rd-stage larvae completed immature development when exposed to 128 ppm of bleach, it is likely that larval development was arrested in the tires from a lack of food. Weekly applications of sublethal doses might have temporarily or partially sterilized the tires. It is also likely that bleach oxidized some organic matter, thereby reducing the amount of substrate available for microbial decomposition.

The treatment described here could be used to prevent the production of *Ae. aegypti* in several types of containers (e.g., used tires, buckets, bottles, flower vases, broken appliances) that residents are reluctant to eliminate from their premises or in cemeteries where flower vases can be abundant. Residential structures such as depressions in floors and roofs, fences, and clogged roof gutters are also candidates for larvicidal applications.

An interesting aspect of the use of bleach to periodically destroy the sources of food for larvae, as opposed to using lethal doses, is the possibility of using the product without inducing inheritable resistance to the compound, because the larvae would die of starvation rather than because of the direct effect of bleach. Advantages of bleach over commercial larvicides are its low price, availability in local stores, and wide use by residents in a variety of household applications. The introduction of bleach as an additional control measure for homeowners is compatible with the recommended approach of source reduction and community participation (Gubler 1989, Gubler and Clark 1994). However, the bleach should not be applied directly or indirectly (spills) to any natural aquatic body of water because most aquatic organisms, including fish, are several orders of magnitude more susceptible to chlorine than *Ae. aegypti* (U.S. EPA 1985). Therefore, if household bleach is eventually approved as a larvicide, it should only be applied for the control of container-breeding mosquitoes.

Some people are already using bleach in their homes to eliminate *Ae. aegypti* larvae (personal observation, Sherman et al. 1998), and the results of this study provide information on the use of appropriate dosages. However, personnel that use this method must remember that conditions vary in artificial containers. Containers with larger amounts of organic matter (e.g., leaf litter) will require a higher dosage of NaOCl than containers with low amounts of organic matter. Thus, whenever a container that produces mosquitoes cannot be eliminated, it would be better to clean it before applying bleach. The combined action of cleaning and bleach will reduce available larval food, will reduce the amount of chlorine required for control of the food and larval instars, and could make the container less attractive for future mosquito oviposition.

ACKNOWLEDGMENTS

We thank Juan F. Medina and Angel Berrios for their assistance with the experiments and an anonymous colleague for useful suggestions.

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