Journal of the American Mosquito Control Association, 15(4):453–457, 1999

CHLORAMINE AND COPPER SULFATE AS CONTROL AGENTS OF PLANKTONIC LARVAE OF *CHIRONOMUS LURIDUS* IN WATER SUPPLY SYSTEMS

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ABSTRACT. The latest approach to control of midge larvae in drinking-water supplies is suppression of the planktonic 1st-stage larvae, by using 2 disinfectants, chloramine and copper sulfate. The median lethal concentration for 24-h exposure of the 1st-stage larvae of *Chironomus luridus* to chloramine and copper sulfate individually was 0.51 and 0.38 mg/liter, respectively. The increase of copper sulfate to 0.5 mg of copper per liter to water containing chloramine (0.5 mg/liter) created a synergistic reaction that resulted in 96% (±8% SD) mortality of the planktonic larvae. This treatment may serve as an effective control of 1st-stage larvae in municipal drinking-water supplies.
KEY WORDS Chironomidae, midge control, water supply, chloramine, copper sulfate

INTRODUCTION

In the early 1990s, the Israeli Water Company began receiving complaints of "red worms" in municipal drinking-water supplies. Investigation of the water distribution system revealed that a majority of the covered storage tanks receiving Lake Kinneret (Sea of Galilee) water via the Israeli National Water Carrier were infested with chironomid midges, mostly of the genus *Chironomus* (Freidberg et al. 1995). The infestation involved all developmental stages and the populations peaked during the summer and autumn. Infestation of drinking-water systems had been previously reported in Germany (Kruger 1941), the United Kingdom (Kelly 1955, Williams 1974), and the USA (Bay 1993, Alexander et al. 1997). Apparently the problem is more widespread than previously recognized in the scientific literature. In the United Kingdom, for example, where water is taken from rivers, chironomid infestation in drinking-water distribution systems is quite common (P. van Poppelen, personal communication).

Adult chironomids are medically important because they cause allergic reactions (Cranston 1995). The larvae have not been found to cause disease or other deleterious effects in humans (Gerardi and Grimm 1982; Ali 1991, 1995). However, these larvae are serious water-quality nuisances in manmade lakes (Mulla et al. 1976) and in municipal potable-water systems.

Broza et al. (1998) described a protocol for efficient control of sedentary last-stage midge larvae in covered storage tanks of the water supply system in Israel by applying high doses of chloramine (shock chloramination) for a short period in temporarily disconnected tanks. In the present study, we examine an alternative approach to the control of the planktonic stage of midge larvae by applying a chronic low concentration of chloramine and copper sulfate. Both these chemicals are permitted for use as control agents in drinking water in Israel. The permissible ranges of chloramine and copper for Israeli consumers are 0.75 and 1.4 mg/liter, respectively (National Health Regulations 1995). Newly hatched midge larvae leave the egg mass and remain planktonic until a suitable habitat for tube building is found (Oliver 1971). We hypothesized that the newly hatched larvae would be more sensitive to chloramine and copper than the other stages and that the 2 chemicals, even in low concentrations, could have a synergistic effect. Control of the planktonic stage would prevent buildup of midge populations.

The effect of copper on chironomid larvae has been relatively well studied (e.g., Rosenberg 1993, Lindegaard 1995). Most of the studies analyzed the effect of copper and other heavy metals on natural and laboratory populations in polluted water. The uptake of copper by chironomid larvae and its fate during metamorphosis were studied by Timmermans et al. (1992), Timmermans and Walker (1990), and Watts and Pasco (1996). The latter showed significant growth reduction of larvae exposed to 2 formulated reference sediments at copper concentrations of 3 and 4 mg Cu^{2+}/liter. Stuijfzand et al. (1996) showed a negative effect of copper at 0.006–0.06 mg/liter on the growth of 1st-stage larvae. Copper hydroxyl and cupric ions, which are the toxic forms of copper, can penetrate into the body through the gut or across the body surface (Koswalt and Knight 1987a, 1987b).

The effect of chloramine on chironomide larvae was not studied before the work of Broza et al. (1998). In invertebrates exposed to chloramine, sublethal physiologic effects include reduction in food filtration rates, decrease in egg production in copepods and rotifers, and decreased rates of respiration and growth in the larvae of the American

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Table 1. Typical chemical composition of tap water used in all the experiments. All concentration values are in mg/liter unless otherwise stated. The chemical analysis of the tap water was performed by the Central Water Quality Laboratory, Mekorot, Nazareth Illit, Israel.

Parameter	Concentration
Ca^+	47
Na^+	115
Mg^+	29.6
Cl^-	236
SO_4^{2-}	56
Br^-	1.5
Cu^{2+}	0.002
CO_2	4.4
Electric conductivity	1,069 μmho/cm (25°C)
Dissolved organic carbon	3.2
Alkalinity	114 mg/liter as $CaCO_3$
Hardness	239 mg/liter as $CaCO_3$
pH	8.1

lobster (Capuzzo 1977, 1979; Beeton et al. 1978). Straub et al. (1995) examined the combined effect of chloramine and copper chloride on *Escherichia coli* and MS-2 coliphage and demonstrated synergism in the inactivation of both organisms. We applied a similar approach to evaluate synergism of chloramine and copper sulfate in order to use low doses of those chemicals as control agents of planktonic chironomid larvae in water-supply systems.

MATERIALS AND METHODS

Laboratory culturing of Chironomus: Egg masses of the dominant chironomid species, identified as *Chironomus luridus* Strenzke, 1959 (Freidberg et al. 1995), were collected from covered storage tanks of the water supply system at Shefar'am, near Haifa, Israel. The eggs were allowed to hatch in dechlorinated tap water and aged for 1 wk; notice that the water chemical composition, shown in Table 1, presents high hardness–alkalinity values. The larvae were kept in the laboratory in screened, 4-liter plastic aquariums containing washed and sterilized sand on the bottom, under 23 ± 2°C and a photoperiod of 14:10 h light: dark. The water was renewed every other day and 0.5 g of food (a mix of 5:1 by weight of Brekies cat food [Effen Producto Eldorado do Sul, Brazil] and yeast extract) was added. Under these conditions, generation time of the midge was about 4 wk. The adults that emerged in the aquaria mated and laid fertile eggs. Egg masses were transferred to hatching containers.

Experimental conditions and chemicals: All experiments were conducted under the above water-temperature, light, and water-quality conditions. A stock solution of monochloramine of approximately 300 mg/liter was prepared (in pH 8.2) by mixing sodium hypochlorite and ammonia in the ratio of 3:1 (w/w) of Cl_2 to N (White 1972) and stored up to 2 wk at 4°C in a dark bottle. Monochloramine residual concentration was determined before use of the chloramine stock solution by the N, N-Diethyl-*p*-phenylenediamine colorimetric method (American Public Health Association 1992). A 1,000-mg/liter copper stock solution of $CuSO_4{\cdot}5H_2O$ was prepared daily. Test concentrations were prepared by diluting appropriate aliquots of the stock solutions with test water.

Bioassay procedure: Bioassays were conducted using newly hatched larvae (<18 h old). Chloramine and copper sulfate were tested individually and in combination. Each exposure consisted of 5 experimental concentrations and 1 control of dechlorinated tap water only. The experiments were conducted in plastic (polyvinyl chloride) scintillation tubes containing 5 ml of the final solution. Each concentration was repeated 5 times with 10–15 larvae in each tube. To minimize loss of chloramine, tubes were tightly closed and wrapped with Parafilm (American National Can, Neenah, WI) on top. The larvae were exposed to the chemicals for 24 h, after which the dead and surviving larvae were counted. Larvae that did not move when stimulated with a Pasteur pipette were counted as dead.

Data analysis: Mortality in laboratory bioassays was corrected for control mortality, and the data were analyzed by log-dose-probit regression (U.S. Environmental Protection Agency 1988). Synergism was determined following Berenbaum's (1985) formula modified for disinfection kinetics by Kouame and Hass (1991):

$$\sum_{i=1}^{n} x_i/y_i = 1,$$

where x_i = concentration of the individual agent in the combination, y_i = concentration of the agent that individually would produce the same magnitude of effect as that of the combination, i = individual agent, and n = total number of agents. The equation is interpreted as follows: Σ values < 1 indicate synergism; Σ values > 1 indicate antagonistic interaction; additivity is obtained when Σ = 1 (i.e., zero interaction).

RESULTS

The concentrations of chloramine and copper sulfate that caused mortality of one half of the 1st-instar population in 24 h were 0.51 and 0.38 mg/liter, respectively. These concentrations were within the permissible range for Israel. The 90% lethal concentration value for copper alone was also within the permissible range, but that for chloramine was not (Table 2).

The effects of increasing concentrations of chloramine (from 0.3 to 0.5 mg/liter) and copper sulfate (from 0.1 to 0.3 mg Cu/liter) and their combination are shown in Fig. 1. The mortality of the larvae exposed to the mixture of chloramine and copper

Table 2. Sensitivity (LC_{50}, LC_{90}) of *Chironomus luridus* 1st instars exposed for 24 h to chloramine and copper sulfate (n = total number of larvae).[1]

	n	LC_{50} (mg/liter)	(95% CL)	LC_{90} (mg/liter)	(95% CL)
Chloramine	300	0.51	(0.39–0.62)	1.63	(1.2–2.9)
Cu as copper sulfate	300	0.38	(0.26–0.49)	1.2	(0.86–2.43)

[1] LC_{50}, median lethal concentration; LC_{90}, 90% lethal concentration; CL, confidence limits.

was always higher than that observed for each chemical alone. Synergistic interaction was tested following Kouame and Hass (1991). The regression equations for calculating y_i concentrations (log-dose-probit regression analysis; U.S. Environmental Protection Agency 1988) for chloramine and copper sulfate were $y = 2.55x + 5.74$ and $y = 2.53x + 6.08$, respectively, where y = probit mortality and x = log concentration. The concentration ratios ($\Sigma x_i/y_i$) that assess the interaction between the chemicals are shown in Table 3. In all combinations, the results indicated synergism. An average mortality of 90% (±10% SD) was obtained in the above-mentioned bioassays when chloramine and copper were used in a combination of 0.5 and 0.3 mg/liter, respectively. When copper concentration in the mixture was increased to 0.5 mg/liter, larval mortality reached 96% (±8% SD).

DISCUSSION

Chloramine and copper have adverse effects on freshwater invertebrates. Chloramine median lethal concentration (LC_{50}) values ranged from 0.03 for 14-day-old amphipods (*Hyalella azteca*) (Ingersoll et al. 1995) to 2 mg/liter for the 4th instar of the chironomid *C. luridus* (Broza et al. 1998). Copper LC_{50} values ranged from 0.01 for 7-day-old oysters (*Crassostre viginica*) to 0.32 mg/liter in the copepod *Acartia tonsa* (Cappuzo 1979). Chironomids, including *C. luridus,* seem to be relatively less sensitive to copper than most other freshwater species (Table 4).

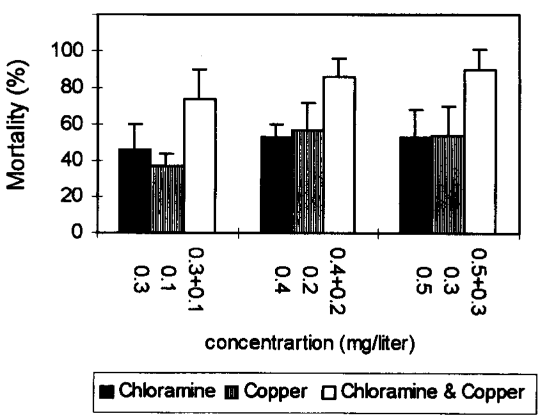

Fig.1. Comparison of mortality rates (mean ± SD) of 1st instar *Chironomus luridus* exposed for 24 h to 3 different concentrations of chloramine, copper, and a combination of both chloramine and copper.

In an earlier study (Halpern 1997 and Table 4), we showed that 1st instars of *C. luridus* are 9 times more sensitive to copper than 4th instars after 24 h of exposure. Gauss et al. (1985) found that the sensitivity of 1st instars of *Chironomus tentans* Fabr. exposed for 96 h to copper is 12 times higher than that of the 4th instar (Table 4). Water hardness as well as the stage affects the sensitivity of *Chironomus* larvae to copper. The median effective concentration value for 1st instars in hard water (172.3 mg/liter as $CaCO_3$) is 6 times higher than in soft water (42.7 mg/liter as $CaCO_3$) (Gauss et al. 1985). The water composition at our site (Table 1) reveals especially high hardness–alkalinity values; thus, our proposed treatment in soft water could be even more effective.

The concentration causing 50% mortality of 1st-instar *C. luridus* exposed to either of the chemicals was within the permissable range for both chemicals in drinking water in Israel. However, 50% mortality is insufficient for effective prevention of re-establishment of midge populations to nuisance levels. Addition of copper at 0.5 mg/liter to water that already contains chloramine at 0.5 mg/liter for disinfection purposes resulted in a synergistic effect, and the mortality of 1st-instar midges increased to above 90%.

Broza et al. (1998) suggested a protocol for controlling *Chironomus* benthic larvae, which are the pestiferous stage, by using a high concentration of chloramine applied for a short period of time. In the present study, a low concentration of chloramine and copper sulfate in a mixture caused more than 90% mortality of 1st instars. This would enable an effective control of the midge infestation in

Table 3. Interaction analysis of the combined mortality effect on 1st-instar *Chironomus luridus* larvae to 24 h of exposure to chloramine and Cu, as copper sulfate, in different concentration combinations.

Chloramine concentration (mg/liter)	Copper concentration (mg/liter)	Concentration ratio ($\Sigma x_i/y_i$)	Interaction
0.3	0.1	0.48	Synergism
0.4	0.2	0.51	Synergism
0.5	0.3	0.4	Synergism

Table 4. Lethal concentrations of copper to various *Chironomus* species.

Species	Life stage	LC_{50}[1] (mg/liter)	Duration	Reference
Chironomus luridus	1st instar	0.38	24 h	Present study
Chironomus luridus	4th instar	3.40	24 h	Halpern 1997
Chironomus decorus	4th instar	0.74	48 h	Koswalt and Knight 1987a
Chironomus tentans	1st instar	0.098	96 h	Gauss et al. 1985
Chironomus tentans	4th instar	1.18	96 h	Gauss et al. 1985
Chironomus tentans	2nd and 3rd intars	0.054	10 days	Ingersoll et al. 1995

[1] LC_{50}, median lethal concentration.

potable-water supply systems. The control of planktonic larvae in drinking-water supplies has not yet been evaluated under field conditions. Although data presented here refer to *C. luridus,* we suggest testing the use of these nonselective control agents (Wolfe at al. 1984, Chen 1995) on other *Chironomus* and Chironomini species as well. The suggested method can be useful for distribution systems that are subject to repeated infestation from either outside or inside sources (e.g., parthenogenic species that can reproduce within the system; Alexander et al. 1997).

ACKNOWLEDGMENTS

This research was supported by grants from Mekorot Water Co., the National Water Company, Israel. We greatly appreciate the support and encouragement of Jacob Zack and the late Jacob Eren. Many thanks go to Dina Poliakov for excellent assistance. The comments made by P. van Poppelen (Kent, United Kingdom) on an earlier version were much appreciated.

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