DETERMINATION OF LEAD TOXICITY IN *CULEX QUINQUEFASCIATUS* MOSQUITOES IN THE LABORATORY

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Abstract. Laboratory investigations were carried out to study the effects of lead toxicity and lead uptake on *Culex quinquefasciatus* mosquitoes. Three different concentrations of lead nitrate were used in laboratory tests (0.05, 0.1, and 0.2 mg/l). An atomic absorption spectrometer (AAS) was used to the determine lead concentrations. The results showed that lead significantly reduced hatching, egg-production, and emergence rates, compared with the unexposed group ($p<0.05$). The ratio of female to male offspring was 3.64:1, which was observed in the second generation, after the parents were exposed to 0.2 mg/l lead. No effects were observed on oviposition preference, larval weight, or larval deformation. The LC_{50} of lead against *Cx. quinquefasciatus* larvae within 24 hours was 0.18 mg/l. There was a significant increase in lead uptake related to increased lead exposure in mosquito larvae ($p<0.05$). The bioconcentration factor (BCF) showed that the lead concentration in the larvae was 62 times greater than in the water. The lead concentration from parents to offspring reduced in the first and second generations ($p<0.05$). There was no significant difference between female and male mosquitoes in lead concentration ($p>0.05$).

INTRODUCTION

Lead is one of the most toxic metals for aquatic organisms (Body *et al*, 1991) and plays an important role in water and air contamination. This heavy metal can accumulate in the human body and have an impact upon human health. Any form of lead is highly toxic. The effects are usually expressed after accumulation in the body over a period of time.

Lead has been found in water sources in Bangkok (Pongpritra and Suan-arounsawat, 1998). A few papers in Thailand have reported heavy metals in water, sediment, and various tissues of some fish species (Tangam, 2000; Chaiyabutr, 1997). Lead was detected in cockles (*Anadara granosa*) in Samut Prakan and Chon Buri Provinces (Muangdech, 2002). The toxicity of lead to aquatic organisms and its accumulation in water and the aquatic biota are well documented, but only a few involve insects (WHO, 1989). Oladimeji and Offem (1989) investigated the accumulation and toxicity of lead to *Chironomus* larvae and reported a "safe" concentration for larvae of 0.27 mg/l. The toxicity to aquatic organisms of heavy metals has been extensively studied. Some acute and chronic effects on aquatic insects have been noted (Clements, 1992). Research carried out in Italy by Romi *et al* (2000) reported that metallic copper at a concentration of 10 g/l induced high mortality and lack of development in *Aedes albopictus* larvae; doses of 20 g/l and higher completely inhibited larval development.

No study of lead toxicity in *Culex quinquefasciatus* mosquitoes has been reported, even though this mosquito is widely distributed in wastewater, is a nuisance, and has a high potential for the transmission of filariasis. This study indicates a potential advantage of using this vector to highlight environmental conditions, since the *Cx. quinquefasciatus* mosquito can take up lead from its water habitat. It is believed that pollutants are ingested by the mosquito larvae because of their feeding habits, in addition to skin absorption. If the lead were not metabo-

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lized, it would be bioaccumulated. Since mosquito larvae are relatively sedentary and inhabit the substrate, it is believed that measurable quantities of pollutants could be absorbed by the larvae. It has been hypothesized that mosquito larvae have a certain degree of sensitivity to heavy metal pollution.

Lead uptake by *Cx. quinquefasciatus* has not been investigated, and a better understanding can help interpret the results of toxicity tests. Details of the toxicity of lead and its uptake in larvae may explain the survival of this mosquito in wastewater areas, and this nuisance mosquito may be used as an indicator of lead pollution.

MATERIALS AND METHODS

Colonization of mosquito larvae for lead detection

To obtain mosquito larvae free of lead contamination for laboratory testing, the food, fourth-stage larvae, adult mosquitoes and water were checked for lead contamination by atomic absorption spectrometer (AAS, Hitachi Model Z-8200; graphite furnace) prior to assay.

Two types of dog food were tested for lead contamination: 1) Apro beef flavor, currently used in the routine laboratory, and 2) Pedigree brand chicken/egg formula, Thailand. Both were randomly purchased from a supermarket.

Two types of dog food and two types of water (distilled and tap water) were provided for the first- to fourth-stage mosquito larvae. The fourth-stage larvae and adult mosquitoes were collected to detect lead contamination after feeding with different types of food.

The conditions for maintaining mosquito larvae as a control, types of water and food to be used, were selected to obtain the least lead contamination in the food, fourth-stage larvae, adults and water.

Determination of lead toxicity in *Cx. quinquefasciatus* mosquitoes

Lead toxicity in *Cx. quinquefasciatus* was determined for oviposition preference, egg hatching, larval development including deformation, mortality rate in each stage, emergence rate, period of development, LC_{50}, and sex ratio after emergence. Experiments were carried out from February 2003 to May 2004 at room temperature (25±2ºC) and a photoperiod of 12:12 (L:D) hours.

Oviposition assay. A small plastic container (10 cm in diameter) was filled with 100 ml of distilled water and assigned as a control, whereas another three containers were filled with different concentrations of lead (0.05, 0.1, and 0.2 mg/l lead in distilled water). A set of four containers with different lead concentrations was put into a cage of 100 gravid females. The assay was performed in duplicate. Egg rafts were counted under a dissecting microscope. This experiment was designed to observe the preference of *Cx. quinquefasciatus* mosquitoes for laying eggs in various concentrations of lead in water.

Egg hatching. Egg rafts were collected from the oviposition assay and transferred to plastic trays containing various concentrated solutions (0, 0.05, 0.1, and 0.2 mg/l) of lead. The trays were covered with plastic sheets to prevent adult dispersion. Tests were carried out in duplicate. Forty-eight hours after hatching, the numbers of hatched larvae were counted and the hatching rate was calculated as the percentage of hatched larvae to the total number of eggs.

Larval development assay. According to the Pollution Control Department (2003), the maximum concentration of lead allowable in industrial effluent is 0.2 mg/l. Therefore, this concentration was selected as the working condition for observing larval development in the laboratory. One hundred newly hatched larvae were placed in a plastic tray containing 750 ml of solution containing 0.2 mg/l of lead. One hundred larvae in the control group were placed in a plastic tray containing the same volume of distilled water. The larvae were fed with dog food daily. Larval stages were checked and counted, and dead larvae were removed. The emergence rate and emergence period (from L1-adult), percentage of mortality in each developmental stage, color change, and mouth-part deformities and twists in morphology of the larvae, were observed. Larvae were weighed before digestion. Adults were counted and the proportion of males to females was reported. The experiments were

repeated from the parents and continued for two generations.

LC_{50} of lead against *Cx. quinquefasciatus* larvae within 24 hours. One hundred newly hatched first-stage larvae were placed in plastic cups containing solutions of 0.1, 0.15, 0.2, and 0.25 mg/l of lead. Twenty-four hours after the first-stage larvae were exposed to lead, the number of dead larvae were counted and reported as mortality and survival rates. A probit/log transformation program was used to determine the LC_{50} of lead against *Cx. quinquefasciatus* larvae within 24 hours. The LC_{50} was calculated from the formula; Y=ax+b where, a=intercept and b=slope.

Determination of lead uptake in *Cx. quinquefasciatus*

A group of 350 mosquito larvae were maintained separately in three lead concentrations (0.05, 0.1, and 0.2 mg/l lead), and in distilled water as the unexposed control group. After seven days, the fourth-stage mosquito larvae and the water in the container were collected and the lead concentrations determined by atomic absorption spectrometer (AAS). Triplicate samples were performed at each concentration. The lead uptakes of the mosquito larvae and the water were compared.

Investigation of lead transfer from parent mosquitoes to offspring

A group of 350 mosquito larvae were separately maintained in three lead concentrations (0.05, 0.1, and 0.2 mg/l lead) and in distilled water as the unexposed control group. After seven days, the fourth-stage mosquito larvae and the water in the container were collected and the lead concentrations determined for the parents. Egg rafts were transferred to new containers filled with distilled water. Lead concentrations were determined in these fourth-stage larvae and observations were continued for two generations where distilled water was used throughout. Triplicate samples were performed for each concentration.

Comparison of lead uptake in male and female mosquitoes

A group of 350 mosquito larvae were maintained separately at three lead concentrations (0.05, 0.1, and 0.2 mg/l), and in distilled water as the unexposed control group. Male and female adult mosquitoes were collected separately after emergence, and lead concentrations were determined for each sex.

Data analysis

Lead measurements in larval and adult mosquitoes were recorded, calculated by wet weight, and reported in μg/g in mosquito larvae and μg/ml in water samples (ppm). Means and ranges were reported in duplicate. Means and SDs were reported in lead uptake and transfer. The Kruskal Wallis test was performed to compare mean oviposition rates, wet larvae weights, mean wet weights between male and female mosquitoes, mean lead uptake in mosquito larvae, mean lead transfer from parent to offspring, and mean lead uptake between males and females in each exposed group, and in the control group. The Mann-Whitney *U* test was performed to compare means of adult weights and means of lead uptake between females and males in each exposed lead group, and in the control group. The chi-square (χ^2) test was performed to compare percentage hatching rates, emergence rates and proportions of sexes among generations in the lead-exposed and control groups. The Z test was performed to compare the emergence rates and proportions of sexes, between the control and the 0.2 mg/l lead-exposed group. Statistical significance was assigned at $p \leq 0.05$.

RESULTS

A preliminary study was performed on the colony of mosquito larvae in order to obtain lead-free mosquitoes for laboratory testing. The results are shown in Table 1. The food selected was chicken/egg flavor dog food, and distilled water was used throughout the study, including maintenance of the control group larvae.

Toxicity of lead against *Cx. quinquefasciatus* mosquitoes

The toxicity of lead against *Cx. quinquefasciatus* mosquitoes was determined for oviposition preference, egg hatching and larval development, including body weight, emergence rate, mortality rate, period of development and

sex ratio after emergence.

Oviposition preference. The females seemed to lay eggs non-specifically in regard to lead concentrations. Of 100 gravid females in the cage, an average of only 41 egg rafts was observed. These egg rafts were distributed in the different lead concentrations. The oviposition preference for each lead concentration is shown in Fig 1. There was no statistically significant difference between oviposition rate and lead concentration ($p>0.05$). The oviposition period ranged from 4-11 days.

Hatching rate. When egg rafts were transferred to new trays of respective lead concentrations and the hatching rate was observed during the period of 6-11 days, it was found that some eggs did not hatch. The hatching rates are shown in Table 2. Statistical analysis showed that the hatching rate for the control group was significantly higher than in the lead-exposed group ($p<0.05$). An estimated 95 L1 per egg raft were found in the unexposed group and an average of 81-96 L1 per egg raft were found in the exposed group.

Larval development. Larval development in this study included emergence rate, period of development (L1 to adult), weight, mortality rate in each stage, deformities, sex ratio after emergence, and LC_{50}.

Emergence rate and period of development (L1 to adult)

The emergence rates in the control group were 78, 86 and 86% for the parents (F0), first (F1) and second (F2) generations, respectively. Larvae maintained in lead-contaminated water were found to have lower emergence rates, 46, 25.5 and 58% for F0, F1, and F2, respectively (Table 3). Statistical analysis by chi-square test showed no significant difference in the emergence rate for each generation ($p>0.05$) in the control group. The emergence rate in the unexposed group was higher than the lead-exposed group, with significant differences in all generations ($p<0.05$).

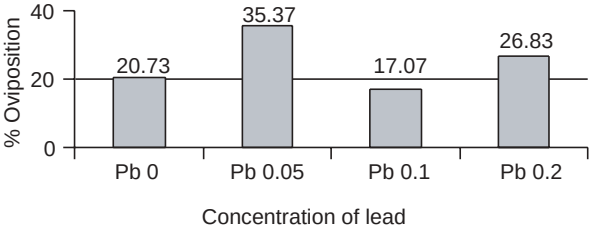

Fig 1–Oviposition preference rate in lead-exposed and unexposed groups. Kruskal Wallis test = 2.625, p=0.453.

Table 1
Preliminary survey of lead concentrations in water, food, the fourth-stage larvae and adult mosquitoes.

Types	Concentration of lead (mean)
Tap water	0.002 μg/ml
Distilled water	UDL
Food	
Beef flavor (in routine lab)	0.09 (range UDL-0.17) μg/g
Chicken/egg flavor	UDL
Fourth-stage larvae fed with different types of food in distilled water	
Beef flavor (in routine lab)	1.05 ± 0.65 μg/g
Chicken/egg flavor	0.02 ± 0.03 μg/g[a]
Adult mosquitoes developed from larvae fed with	
Beef flavor (in routine lab)	UDL
Chicken/egg flavor	UDL

If the assay was run in duplicate, the concentration was reported as the mean (range). If in triplicate, the concentration was reported as the mean ± SD; UDL = under the detectable limits; [a]2 samples where lead was found under detectable limits

Table 2
Percentage hatching rate and mean numbers of L1 per egg raft (fertility).

Conc. of lead (mg/l)	Total No. of egg rafts	No. rafts[a] not hatch	% Hatching[b] (Total no.L1/Total no. eggs)	Mean (L1/egg raft) (Range)
0	17	2	99.36 (1,418/ 1,426)	95 (62-133)
0.05	29	2	94.01 (2,415/ 2,569)	89 (53-147)
0.1	14	1	98.42 (1,246/ 1,266)	96 (50-153)
0.2	22	3	96.06 (1,610/ 1,676)	81 (58-132)

[a]Egg rafts that did not hatch were ruled out (not calculated).
[b]χ^2 =96.47, p=0.000

Table 3
Emergence rate development from a first-stage larva to an adult mosquito.

Group	Emergence rate (range)		
	F0 (%)	F1 (%)	F2 (%)
Control	78 (76-80)	86.0 (84-88)	86.0 (84-88)[a]
0.2 mg/l lead	46 (35-56)	25.5 (21-30)	58.0 (46-70)[b]
Z	4.66*	8.54*	4.41*
P	0.000	0.000	0.000

[a]χ^2 =3.07, p=0.215; [b]χ^2 =21.29*, p=0.000

In terms of time required for larval development, it was found that larvae reared in lead-contaminated water needed a longer time to develop from the L1 stage to adults. The emergence period from a first-stage larva to a first-day adult mosquito took 9-12 days in parents and 9-14 days in the first and second generations of the control group. The emergence in the group exposed to 0.2 mg/l lead was delayed and took longer: 10-14 days for parents and 10-13 days to form adults in the first and second generations, respectively (Table 3).

Mortality rate in each stage

The mortality rate in each stage during larval development is shown in Fig 2. The highest mortality occurred in the first-stage larvae of both the control and lead-exposed groups. However, the group exposed to 0.2 mg/l lead showed a 2.5 times higher mortality for first-stage larvae than in the control group in the parents and in the second generation, and a six times higher mortality rate than in the control group in the second generation. The mortality of the pupal stage was also higher in the lead-exposed group in all generations. Incomplete adult development occurred at a higher rate in the lead-exposed group than in the control group in the parents and in the second generation.

Visible deformations in larval morphology, such as body color, twisted body, and mouthparts were not observed when compared with the controls.

Weight of fourth-stage larvae

The mean weights of the larvae in the control group ranged from 3.23±0.60 to 3.66±0.11 mg per larva and in the exposed groups, the larval weights ranged from 2.85±0.46 to 3.93±0.04 mg per larva. Larval weights were not significantly different between the generations ($p>0.05$), and were not significantly different ($p>0.05$) between the lead-exposed and unexposed groups (Table 4).

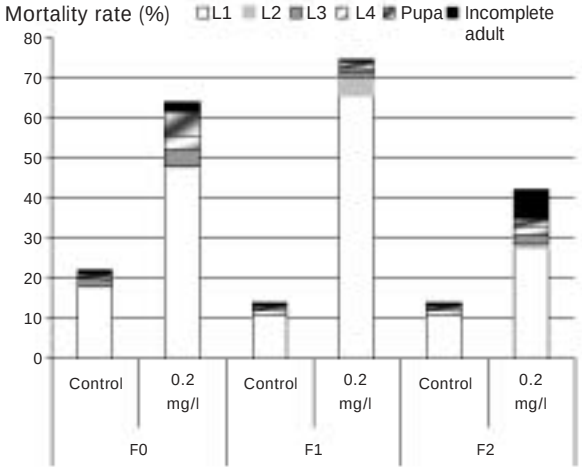

Fig 2–Mortality rate at each stage during larval development.

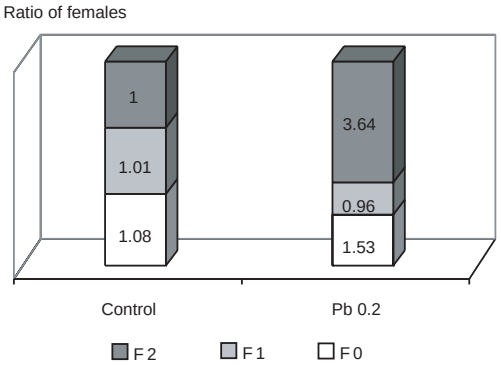

Fig 3–Proportions of female *Cx. quinquefasciatus* in the unexposed and exposed lead groups in the parents and in two generations after adult emergence.

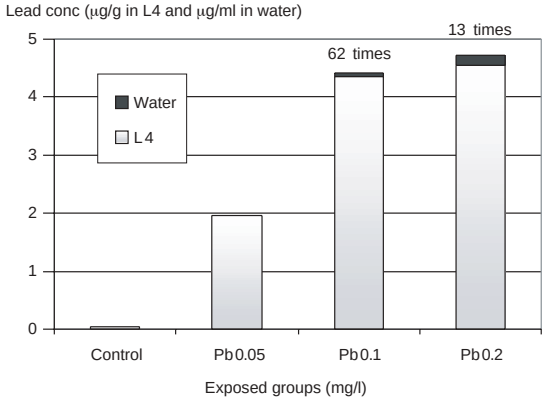

Fig 4–Mean lead uptake in fourth-stage larvae, water (temp 25º+2ºC, pH 7) and BCF.

Proportion of sexes

The results for the sex ratio are shown in Fig 3. It was found that there were more females, especially in F2, where the numbers of females were 3.64 times higher than the males in the exposed group ($p<0.05$).

LC_{50} of lead against *Cx. quinquefasciatus* larvae within 24 hours

Approximately 50% of first-stage larvae survived at a lead concentration of 0.2 mg/l, and more than a 50% mortality rate occurred in concentrations above 0.2 mg/l lead. The results are shown in Table 5.

The LC_{50} for lead against *Cx. quinquefasciatus* larvae within 24 hours was calculated with the probit/log transformation program. In this study, intercept (a)= 5.158787 +/- 8.332521 E and slope (b) = 9.319638 +/- 2.890115. Therefore, the LC_{50} for lead against *Cx. quinquefasciatus* larvae within 24 hours for this experimental condition was 0.18 mg/l (range 0.14-0.24).

Uptake of lead in fourth-stage larvae, water and BCF

In the fourth-stage larvae, lead uptake seemed to increase with higher lead concentrations. The highest mean lead uptake was 4.54±0.22 μg/g in the group exposed to 0.2 mg/l lead. There was a significant increase in lead uptake following concentrations of lead exposure ($p<0.05$) in mosquito larvae, except in the group exposed to 0.1 and 0.2 mg/l lead ($p>0.05$). The results are shown in Fig 4.

Bioconcentration factor (BCF), [C(larvae)/C(water)] showed a higher lead uptake in the larvae than in the water. The BCF was 13-62 ml/g at temperatures of 25º-27ºC, and a pH of 7. Lead levels in the control group and in the 0.05 mg/l group were under detection limits in water, but the larvae showed some lead concentration (Fig 4).

Lead transfer from parents to their offspring

Lead levels decreased from generation to generation in the larva of all the exposed groups. The results are shown in Fig 5. Lead was significantly higher in the parents than in the first and second generations ($p<0.05$).

Table 4
Mean weight of *Cx. quinquefasciatus* larvae in the three generations.

Conc. of lead (mg/l)	Mean weights (mg)/larva			Kruskal Wallis test	p-value
	F0	F1	F2		
Control	3.43±0.43	3.23±0.60	3.66±0.11	3.467	0.177
0.2	3.53±0.96	3.92±0.21	3.93±0.04	0.800	0.670
Kruskal Wallis test	0.746	5.974	7.205		
p-value	0.862	0.113	0.066		

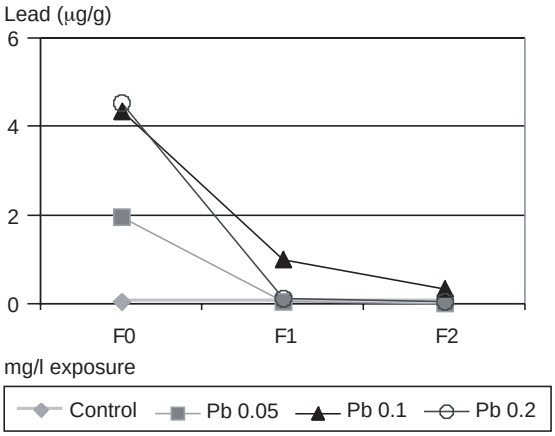

Fig 5–Lead transfer from parents to their offspring determined in fourth-stage larvae (temp 25+2ºC, pH 7).

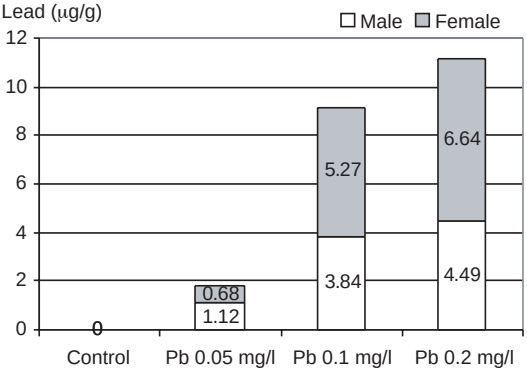

Fig 6–Mean lead concentrations in female and male mosquitoes.

Lead uptake by male and female mosquitoes

There was no significant difference in lead concentrations between the females and males in each lead-exposed group (Fig 6).

Table 5
Mortality rates at different lead concentrations, 24 hours after hatching.

Lead-exposed (mg/l)	Mortality rate (%)
0.1	2
0.15	30
0.2	50
0.25	98

DISCUSSION

It is important to elucidate the conditions for larval colonization to study lead toxicity and lead uptake in mosquito larvae. Therefore, this study considered some factors that may influence laboratory testing. The investigations of lead in food and water for feeding and culturing mosquito larvae were completed before the laboratory setting. Due to the possibility that lead contamination of larvae may derive from food or water, this study endeavored to avoid lead contamination before testing. However, measurable amounts of lead were found in some samples. Purification of samples is difficult because lead contamination is everywhere. This study selected a chicken/egg dog food formula as the food of choice and distilled water as the water habitat because they had minimal lead contamination (Table 1).

Some mosquitoes can survive in polluted wastewater. The toxicity of some heavy metals against these mosquitoes is not yet known. This study was designed to obtain baseline data on lead toxicity in *Cx. quinquefasciatus* in the laboratory.

Female mosquitoes laid their eggs in distilled water and various concentrations of lead solution, and egg-laying started 4-11 days after blood feeding (Fig 1). This study noted the same non-specific preference of oviposition in *Cx. quinquefasciatus* as Romi *et al* (2000) reported; the presence of metallic copper in water did not affect the oviposition of *Aedes albopictus*. Bruno and Lawrence (1979) presumed that priming of the egg raft was enhanced by other rafts laid in the same bowl. *Cx. quinquefasciatus* characteristically lives in polluted water, so the responses of gravid females to a combination of oviposition aggregate with pheromones and polluted water was investigated. Additional effects were obtained with combinations of synthetic pheromones and 3-methylindole, a highly active constituent of some polluted waters (Millar *et al*, 1994). The sensitivity of the antennae of gravid *Cx. quinquefasciatus* females to the pheromones was demonstrated by recording electroantennograms (EAGs).

The hatching rate in the control group was significantly higher than in the lead-exposed groups (Table 2). The number of larvae per egg raft was found to be higher in the unexposed group than in the exposed groups. Romi *et al* (2000) showed that the presence of different doses of metallic copper in the water did not affect egg hatching, and no significant difference was detected between groups and the controls. No studies have reported the effects of lead in the water on egg hatching in *Cx. quinquefasciatus* mosquitoes. Schmidt *et al* (1991) studied the long-term effects of metals (mercury, cadmium, and lead) in the soil on the development of *Aiolopus thalassinus* (Orthoptera, Acrididae). The hatching rate for nymphs developed from eggs laid in treated soil was significantly reduced. The WHO (1989) reported that lead is present on the egg surface, but is not accumulated in the embryo.

The emergence rates were significantly decreased in the groups exposed to 0.2 mg/l in all three generations, including in the parents (Table 3). The first generation seemed to have a higher mortality rate than the other generations, which may be due to the effects of accumulated toxic lead from the parents on the first generation resulting in developmental failure. However, survival adaptation may have occurred in the second generation, leading to higher emergence rates. The time from emergence to adulthood was longer in the lead-exposed groups. Detoxification in insects is poorly understood, but in general, two detoxification systems appear to be involved. One system participating in the detoxification of metals in insects involves the hemocytes (Heliövaara and Väisänen, 1993). Brief exposure of *Chironomus riparius* larvae to equivalent doses (mg/l) of cadmium suggested that exposure to a high concentration for a short time resulted in reduced adult emergence, compared with exposure at a lower concentration for a long time (McCahon and Pascoe, 1991). Insect development involves distinct stages (Gordon, 1984).

The mortality rate in the first-stage larvae of the exposed group was approximately 2.5-6 times higher than the control group (Fig 2). This is similar to the results reported by Romi *et al* (2000), who found that *Ae. albopictus* larval development was delayed and high larval mortality was induced in the first and second generations when exposed to 10 and 20 g/l of metallic copper. They also reported that the eggs of *Ae. albopictus* were not affected by some doses of copper ions, but the first and the second instars were most sensitive to the toxic action of copper. Schmidt *et al* (1991) studied the long-term effects of metals (mercury, cadmium, and lead) in the soil on the development of *Aiolopus thalassinus* (Orthoptera, Acrididae). The mean durations of the F1 and F2 (first and second-offspring generation nymphal stages) were prolonged in all mercury and cadmium treatments and in the treatments with high lead levels (250 to 500 μg/g).

Larval weights were not significantly different between the exposed and unexposed groups (Table 4), contrasting with the report by Romi *et al* (2000), which stated that higher doses of copper resulted in lower adult weights. A number of laboratory studies on *Onchiurus armatus* showed that high levels of lead or copper, ingested via polluted fungi, increased mortality and reduced growth, reproduction and population growth (Bengtsson *et al*, 1983, 1985, 1989).

There were higher numbers of females than

males (1.53 times in the parents and 3.64 times in the second generation lead-exposed groups) (Fig 3). This may imply that females were better able to survive in metal-polluted water. Therefore, transmission of filariasis may become a point of concern. In the first generation, the ratio of the sexes was similar between the exposed and unexposed groups. This may be due to the toxic effects of lead occurring in both sexes, causing the highest mortality in this generation, while females in the second generations had a higher tolerance than the first generation, and the mortality rates in adults were also reduced. In contrast to this study, Heliövaara and Väisänen (1993) found that chlorpyrifos shifted the sex ratio of offspring toward fewer females in *Aphytis melinus* (Aphelinidae). The sex ratio in this study was 1:1 in the lead-unexposed group. Gómez *et al* (1977) also found that the estimated percentage of female adults of *Cx. pipens fatigans* was 54%. Qutubuddin (1952), in an experimental population based on 40 ovipositions, 44.22% were adult females. He reported that in the majority of the hatchings from a single egg mass, the male/female ratio was close to 1. Scorza (1972) concluded that the sex ratio was very close to 1 in the population of *Cx. p. fatigans*.

Deformities, twists in the body, color changes in the body, or mouth-part deformities, were not seen in this study. Pollutants have been reported to cause structural deformities in several insect orders (Theiling and Croft, 1989). The abnormalities included blackening of the cuticle, twists in the body and partially molted cuticles. The frequency of mouth-part deformities in *Chironomus* specimens collected from unpolluted waters was 0.09% (Warwick, 1980). By comparison, the incidence of such deformities in *Chironomus* from polluted waters reached 25-38% in an urban canal in West Berlin (Koehn and Frank, 1980), 77% in Parry Sound Harbor, GA (Hare and Carter, 1976) and 83% in the inner harbor area of Port Hope Harbour, Ontario (Warwick *et al*, 1987). This sublethal alteration process may take a long time, and will result in an acute effect on the mosquitoes, because it takes 7-14 days in a water habitat. This study found that a concentration of lead >0.25 mg/l completely inhibited larval development and killed all larvae.

The LC_{50} for lead against *Cx. quinquefasciatus* larvae within 24 hours was found to be 0.18 mg/l. These data have not been reported elsewhere. Oladimeji and Offem (1989) investigated the accumulation and toxicity of lead on *Chironomus tentans* larvae and indicated that *Chironomus* larvae were the most tolerant to lead poisoning. The "safe" concentrations of lead for Chironomid larvae were 0.27 mg/l, whereas the LC_{50} for Capitella larvae at 96 hours was >1.2 μg/l (Reish *et al*, 1976), for the water flea, *Daphnia magna*, at 48 hours, it was 0.45 μg/l (Biesinger and Christensen, 1972), for the midge larvae, *Tanytarsuss dissimilis*, at 10 days it was 0.258 μg/l (Anderson *et al*, 1980), for the mayfly larvae, *Eperella grandis*, at 14 days it was 3.5 μg/l and in the stonefly larvae, *Pteronarcys californica*, at 14 days it was >19.2 μg/l (Nehring, 1976). Anderson *et al* (1980) also exposed the chironomid midge, *Tanytarsus dissimilis*, to lead nitrate during two different stages of its lifecycle and found that the average LC_{50} from two tests was 0.258 mg/l.

The uptake and accumulation of lead by aquatic organisms from water and sediment are influenced by the various environmental factors, such as temperature, salinity, and pH, as well as humic and alginic acid content (WHO, 1989). The fourth-stage larvae of *Cx. quinquefasciatus* mosquitoes showed increasing lead levels with increased lead exposure concentrations. In the control group, lead contamination in the fourth-stage larvae was very small, and may have come from the food.

The results of this study show a positive correlation between lead exposure and lead uptake in mosquitoes (Fig 4), which is similar to the study of lead accumulation in *Ipomoea aquatica* Forsk and *Neptunia oleracea* Lour from surface water where accumulations increased with the exposure doses (Phuvachiranon, 1993). However, the uptake of lead by *Pomphorhynchus laevis* cystacanths in *Gammarus pulex* and immature worms in chub (*Leuciscus cephalus*) were found to increase in a dose-related manner (Siddall and Sures, 1998). The study by Kay *et al* (1984) showed that the accumulation of lead in the water hyacinth (*Eichornia crassipes*) was dose-related. The study by Pringle *et al* (1968)

demonstrated that mature oysters exposed to lead in the water at 25, 50, 100, and 200 μg/l had lead uptakes of 0.35, 0.71, 1.50, and 4 mg/kg per day, respectively. Lead levels in invertebrates reflect dietary concentrations and feeding strategy (Andrews *et al*, 1989).

The bioconcentration factors (BCFs) in the fourth-stage larvae were 13-62 (Fig 4). Vighi (1981) described BCFs in plants and guppys, Nakada *et al* (1979) in plants, Muramoto and Oki (1983) and Kay and Haller (1986) in water hyacinths, Waltling (1983) in oysters,marine mollusks and mussels, Coombs (1977) in mussels, Spehar *et al* (1978) in snails and amphipods, Muramoto (1980) in carp, Merlini and Pozzi (1977) in pumpkinseeds and sunfish, Somero *et al* (1977) in gobys, and Wong *et al* (1981) in rainbow trout. A few studies have been reported in insects. Vighi (1981) studied BCFs in the water flea, Spehar *et al* (1978) studied the caddis fly and stonefly, and Nehring (1976) studied the stonefly and mayfly. Many researchers compared dry weight BCFs, which is different from our study. In our study, higher concentrations (about 4-10 times) were reported. As was noted by Phuvachiranon (1993), the proportions between the wet and dry weights were approximately 4-10 times in plant species. In our study, the wet weights of the fourth stage larvae were seven times higher than the dry weights (800 larvae dried at 70ºC for 24 hours). We measured the lead in the dry weight larvae as five times higher than in the wet weight larvae.

Lu *et al* (1975) established aquatic/terrestrial model ecosystems based on three different soil types. Using silica sand, with a natural lead concentration of 0.122 mg/kg and with 10 mg/kg lead chloride added, the lead levels in organisms were higher than in other soils. With this soil, lead levels were as follows: water 0.013 mg/kg, algae 275 mg/kg, daphnids 187 mg/kg, snails 334 mg/kg, mosquito larvae (*Cx. quinquefasciatus*) 403 mg/kg, fish 13 mg/kg, sorghum leaves 497 mg/kg and sorghum roots 695 mg/kg. Using silica sand with 10% silty clay loam (natural lead content 4.5 mg/kg) and 10 mg lead chloride/kg added, the lead uptake by all the organisms was markedly lower. The lead levels were as follows: water 0.002 mg/kg, algae 114 mg/kg, daphnids 85 mg/kg, snails 56 mg/kg, mosquito larvae (*Cx. quinquefasciatus*) 80 mg/kg, fish 1 mg/kg, sorghum leaves 1 mg/kg and sorghum roots 5 mg/kg. From this model, the lead levels were the highest in the mosquito larvae, and may be regarded as an early monitor of toxic metal contamination in the environment.

Larvae of some mosquito species, such as *Cx. quinquefasciatus* and *Cx. gelidus*, are able to survive in polluted water (Whelan *et al*, 2000). This advantage may lead to the use of mosquitoes to indicate some heavy metal contamination in water in the future. The toxicity of heavy metals in *Cx. quinquefasciatus* has never been studied. This vector is widely distributed around industrial areas. Since many people live around areas where reservoirs for filarial parasites exist, mosquito vectors may spread widely and possess a high potential to transmit disease.

It has been reported that lead is transferred from mother dolphins to their offspring during fetal development and lactation (Honda *et al*, 1986). In this study, lead remained at low concentrations in the first and second generations of the fourth-stage larvae (Fig 5). Perhaps lead uptake occurred in the first-stage larvae until the parents' pupal stage and was excreted during the adult stage without any more lead exposure. In the first and second generations, there was no more larval exposure to lead, so that most of the lead was excreted via the metabolism of the larvae. Lead transfer from parent to offspring may not be possible through many generations. Emergence and molting may be mechanisms that decrease the metal content in the insect (Krantzberg and Stokes, 1988). Van Straalen and Van Meerendonk (1987) suggested that lead in the springtail, *Orchesella cincta*, was present in three compartments; the most important compartment for lead was the digestive tract. Lead in the gut has a half-life of less than one day; the fast body burden only halves within a week, while the slow body burden takes about three weeks. Mayer *et al* (1986) found significant accumulations of lead in the fat, midgut and rectum, while Hare *et al* (1991) reported that cadmium accumulated mainly in the digestive tract of many types of aquatic insects.

Lead in female mosquitoes was not signifi-

cantly different from the males. The proportion of females was higher than males in the second generation exposed to 0.2 mg/l lead, which was significantly different from the unexposed group. The adaptation of females to lead pollution may be greater than males. Finley *et al* (1976) reported females had double the lead levels of male mallards, while Honda *et al* (1986) found more lead in adult male dolphins than in females.

This paper serves as a pilot study and the findings should only be treated as baseline data that can be used to inspire further, more detailed, projects in related areas. *Cx. quinquefasciatus* females from lead areas may have to be screened for filarial parasites since they were numerous in the study area. It would be particularly useful to determine the levels of heavy metals in other mosquito species, because some other heavy metal areas may have diverse mosquito species. It may be of interest to test other heavy metals against mosquitoes, as well.

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