Impact of Inorganic Pollutants Perchlorate and Hexavalent Chromium on Efficacy of *Bacillus sphaericus* and *Bacillus thuringiensis* subsp. *israelensis* Against *Culex quinquefasciatus* (Diptera: Culicidae)

MARY A. SORENSEN,[1] WILLIAM E. WALTON, AND JOHN T. TRUMBLE

Department of Entomology, University of California, Riverside CA 92521

J. Med. Entomol. 44(5): 811–816 (2007)

ABSTRACT The effects of two widespread environmental pollutants, perchlorate and hexavalent chromium, were assessed on the efficacy of *Bacillus thuringiensis* subsp. *israelensis* (Bti) and *Bacillus sphaericus* (Bsph) against fourth instars of *Culex quinquefasciatus* Say (Diptera: Culicidae) in 24-h laboratory bioassays. Although 250 mg/liter perchlorate, a level somewhat higher than would be considered ecologically relevant, did not affect the control provided by either larvicide, presence of 1.04 mg/liter hexavalent chromium, an ecologically relevant concentration, increased the efficacy of both Bti and Bsph by 21 and 80%, respectively. In the presence of hexavalent chromium, improved suppression could be expected from *Bacillus* applications at the current label rates. However, because hexavalent chromium has been shown to affect many taxa, we propose that the potential exists for increased susceptibility of nontarget organisms to *Bacillus* products in polluted habitats.

KEY WORDS ecotoxicology, environmental contaminant, pollution, mosquito, larvicide

Currently, *Bacillus thuringiensis* subsp. *israelensis* (Bti) and *Bacillus sphaericus* (Bsph) are commonly used control agents for mosquitoes because of their specificity, effectiveness, and low risk to humans and the environment (Becker et al. 2003). However, the efficacy of these materials is known to be affected by pollutants. For example, Bsph is considered superior to Bti in habitats heavily contaminated with organic waste (Karch et al. 1991, Amalraj et al. 2000), although newer formulations of Bti suitable for organically enriched habitats are now becoming available (Gunasekaran et al. 2002). The effects of inorganic pollutants on the efficacy of both Bti and Bsph are largely unknown.

Perchlorate (ClO_4^-) is a common and persistent (Urbansky 1998) inorganic pollutant. This chemical is used as an oxidant in rocket fuels, roadside flares, airbags, fireworks, and other combustibles (Sorensen and Trumble 2004). In addition, some perchlorate may be produced by atmospheric processes (Dasgupta et al. 2005). In vertebrates, perchlorate interferes with iodide uptake by the thyroid gland (Wolff 1998). Although federal regulations recommend perchlorate in drinking water be at or below 24 μg/liter (Committee to Assess the Health Implications of Perchlorate Ingestion 2005), protective levels for ecosystems have not been established. Sorensen et al. (2006) showed that perchlorate contamination increased larval mortality and prolonged larval development of *Culex quinquefasciatus* Say (Diptera: Culicidae), an important vector of West Nile virus and encephalitis viruses. These findings are of importance because drinking water or irrigation water in >50% of the United States has been found to be contaminated with perchlorate (U.S. EPA 2005a), and 44 states have sites where perchlorate was manufactured or used at one time (U.S. EPA 2002).

In California, >400 wells and drinking water sources contain perchlorate above the 0.1 μg/liter detection limit; these wells are used extensively for irrigation, which makes the contaminant more bioavailable over large areas. In addition, California contains a number of industrial or military sites with perchlorate contamination, particularly in Los Angeles, San Bernardino, and Riverside counties. In Nevada, the now-closed Kerr-McGee Plant for rocket fuel has been responsible for up to 455 kg of perchlorate leaching daily into the Colorado River (Hogue 2003). This river is a primary source of drinking and irrigation water for several cities in the southwestern United States, including Los Angeles, Las Vegas, San Diego, Phoenix, and Tucson. Mosquitoes can develop in almost any available standing water source, including temporary pools and small containers (Becker et al. 2003). Perchlorate present in small containers will concentrate with evaporation, increasing the considerable potential for mosquito larvae to develop in environments with highly elevated concentrations of perchlorate.

Similarly, water and soil contamination with hexavalent chromium are also significant worldwide problems (http://www.worldbank.org/nipr/polmod.htm). Chro-

[1] Corresponding author, e-mail: mary.sorensen@email.ucr.edu.

mium has been used extensively for many industrial applications, including metal alloys, metal plating, chrome colors and dyes, cement manufacturing, and wood preservatives (Kotas and Stasicka 2000). Although trivalent chromium is an essential trace element for most biota, hexavalent chromium [chromium(VI)] is both toxic and carcinogenic (Zhang and Li 1987, Smith et al. 1989, Elbetieha and Al-Hamood 1997). Federal regulations limit chromium(VI) in drinking water to 0.05 mg/liter (Eisler 1986), and, like perchlorate, effects of chromium on insects are not well understood. Chromium has been demonstrated to have toxicity to some aquatic insects (Vuori and Kukkonen 1996, Leslie et al. 1999, Canivet et al. 2001) and at least one terrestrial detritivore (*Megaselia scalaris;* Trumble and Jensen 2004). Like perchlorate, chromium(VI) was shown to have the potential to cause reduced survival and prolonged development of *Cx. quinquefasciatus* (Sorensen et al. 2006).

In the San Fernando Valley of southern California, the U.S. Environmental Protection Agency's (EPA's) monitoring wells have detected concentrations of chromium as high as 1.0 mg/liter (Los Angeles Regional Water Quality Control Board 2004) and in the eastern part of the state a plume containing >12 mg/liter is nearing the Colorado River (Lifsher 2004). The San Fernando Valley Basin houses and contributes to the Los Angeles Reservoir, the endpoint of the Los Angeles Aqueduct and a major source of water for the Los Angeles metropolitan area. The purpose of this research was to evaluate the potential for perchlorate and chromium(VI) to interfere with or interact with *Bacillus* microbial larvicides.

Materials and Methods

Eggs were collected as needed from a *Cx. quinquefasciatus* colony maintained at the University of California, Riverside, and hatched in a shallow pan (30 by 20 by 5 cm) containing high-performance liquid chromatography (HPLC)-grade water (Milli-Q Water System, Millipore Corporation, Billerica, MA) and ≈5 ml of mosquito food. Food consisted of a 3:1 (wt:wt) mixture of ground mouse chow (mouse/rat diet, Harlan/Teklad, Madison, WI) and brewer's yeast (MP Biochemicals, Aurora, OH) rehydrated with 50 ml of HPLC-grade water to ≈4 g of powdered food. Second instars were transferred to bioassay containers by using an eyedropper. Bioassays were conducted in 100-ml glass jars with plastic lids containing 90 ml of HPLC-grade water. Each test jar was stocked with 20 larvae and given four drops of food and two drops of food daily thereafter. In all trials, each treatment was replicated four times. Because perchlorate was shown to have an effect on *Cx. quinquefasciatus* only at high levels (Sorensen et al. 2006), the perchlorate treatment (250 mg/liter) applied here represents some of the most contaminated sites publicly reported (U.S. EPA 2005b). Hexavalent chromium (1.04 mg/liter) dosage was chosen to be high yet still ecologically relevant (within the range of reported levels). All controls consisted of bioassay jars stocked as described previously but without contaminant added. Bioassay jars were maintained in an incubation chamber at 28 ± 1°C, 75% RH, and a photoperiod of 14:10 (L:D) h.

When larvae reached the fourth larval stadium, they were starved for 24 h. After the starvation period, each jar received 10 ml of HPLC-grade water containing the Bti or Bsph treatment. Concentration levels for Bti (0.25–1.5 μg/liter) and Bsph (0.005–0.08 mg/liter) were chosen to encompass the LC_{50} level for *Cx. quinquefasciatus* without causing 0 or 100% mortality, based on data from preliminary trials (M.A.S., unpublished data). Controls received 10 ml of HPLC-grade water. Mortality was assessed at 24 h, and larvae that did not respond to tapping on the container were counted as dead. Although the standard bioassay for Bsph calls for assessing mortality at 48 h, a 24-h bioassay was used because of high control mortality at 48 h due to the additional stress from contaminant treatments. Each contaminant [perchlorate or chromium (VI)] was combined with at least three concentrations of each pesticide (Bti or Bsph).

Perchlorate was added to bioassay containers as ammonium perchlorate (CAS 7790-98-9, NH_4ClO_4, 99.999%, Aldrich Chemical Co., Milwaukee, WI). Chromium(VI) treatments were created using chromium trioxide (CAS 1333-82-0, CrO_3, 99.8%, Fisher Scientific, Pittsburgh, PA). In water, ammonium perchlorate dissociates readily; concentrations are reported here as concentration of ClO_4^-. Chromium trioxide dissolved in water will form chromic acid (H_2CrO_4), but it also may undergo a number of other reactions, including complexing with dissolved organic and inorganic molecules. Chromium(VI) is therefore reported here as concentration of elemental chromium. *Bacillus thuringiensis* subsp. *israelensis* treatments were created using the commercial Bti product Gnatrol (lot V01B-600FL-1, 600 international toxic units [ITU]/mg, Valent Biosciences Corporation, Libertyville, IL). *Bacillus sphaericus* treatments were created using the Vectolex technical powder (lot 117-140-W5-01, 1483 ITU/mg, Valent Biosciences).

To determine whether contaminant treatment significantly altered the effect of Bti or Bsph on mosquitoes, we performed an analysis of covariance (ANCOVA) (SAS Institute, Cary, NC; www.sas.com) with log dose of Bti or Bsph as the covariate, treatment as the type of contaminant added (chromium, perchlorate, or control), and mortality (expressed as the probit transform) as the dependent variable. To determine the LC_{50} values and associated standard errors, probit analyses were used (Minitab, Inc. 2000).

Results

***B. thuringiensis* subsp. *israelensis*.** Even at the exceptionally high rate of 250 mg/liter, perchlorate contamination did not change the efficacy of Bti at 24 h postexposure against *Cx. quinquefasciatus* (Fig. 1A) (ANCOVA: $F_{1,17} = 3.13$; $P = 0.097$). This lack of effect was consistent with our previous studies, indicating that perchlorate has minimal impact on mosquito

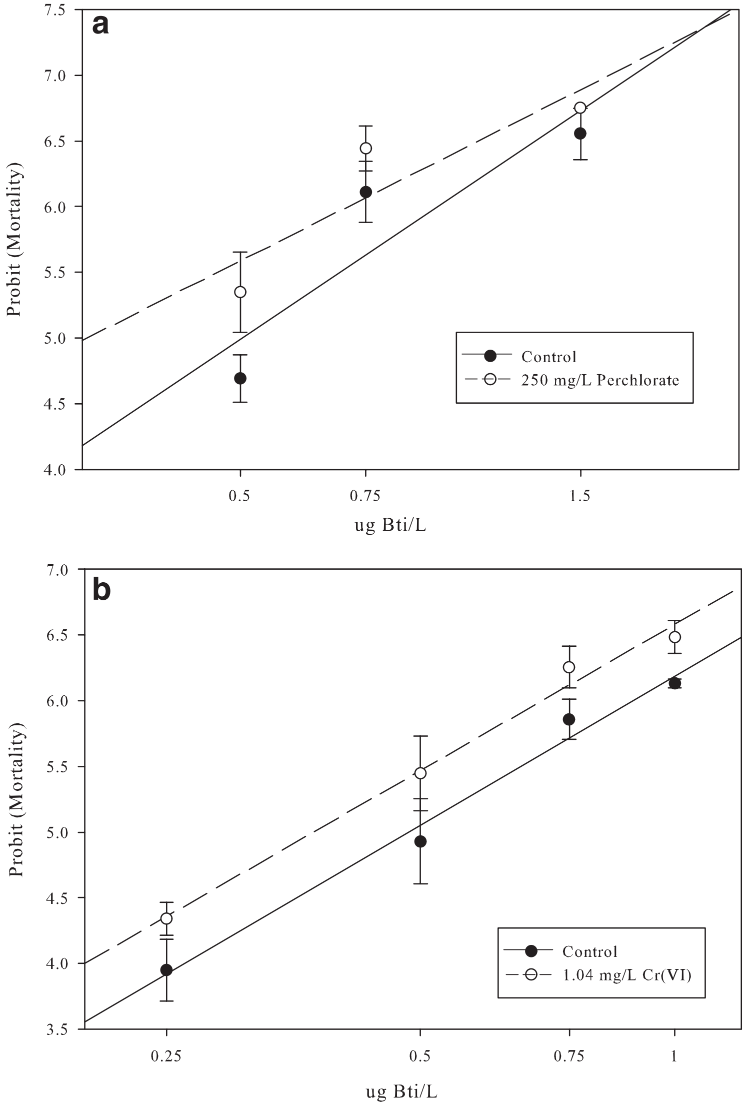

Fig. 1. Mortality of fourth instars of *Cx. quinquefasciatus* reared in water contaminated with 250 mg/liter perchlorate (a) or 1.04 mg/liter hexavalent chromium (b) and then treated with Bti. Dose of Bti is plotted on a log scale. Vertical bars represent SE.

growth and survival unless ecologically meaningful concentrations are exceeded (Sorensen et al. 2006).

In contrast, larvae reared in 1.04 mg/liter chromium(VI) were significantly more susceptible to Bti than control larvae (Fig. 1B) (ANCOVA: $F_{1, 29} = 10.98$; $P < 0.001$). The ANCOVA model did not include a significant interaction term, indicating that the probit lines are parallel and the effect of pollutant was equal across concentrations of Bti. The LC_{50} value for chromium (VI)-exposed larvae decreased by 21% compared with the control group LC_{50} (Table 1). Given that chromium(VI) occurs in the environment at levels exceeding those tested in this study (Kotas and Stasicka 2000), and this pollutant causes reduced growth and increased mortality in *Cx. quinquefasciatus* larvae at even 0.16 mg/liter (Sorensen et al. 2006), an increase in the efficacy of Bti may be expected.

B. sphaericus. Similar to the results obtained with Bti, 250 mg/liter perchlorate did not change the toxicity of Bsph to *Cx. quinquefasciatus* larvae (Fig. 2A)

Table 1. Activity of *Bacillus* microbial control agents against fourth instars of *Cx. quinquefasciatus* reared in water contaminated with perchlorate or hexavalent chromium

	B. thuringiensis var. *israelensis*			*B. sphaericus*		
	n	LC_{50}	SE	*n*	LC_{50}	SE
Control	9	0.532	0.027	17	0.019	0.002
250 mg/liter Perchlorate	9	0.410	0.039	18	0.010	0.002
Control	15	0.478	0.025	17	0.019	0.002
1.04 mg/liter Chromium(VI)	15	0.377	0.023	18	0.003	0.002

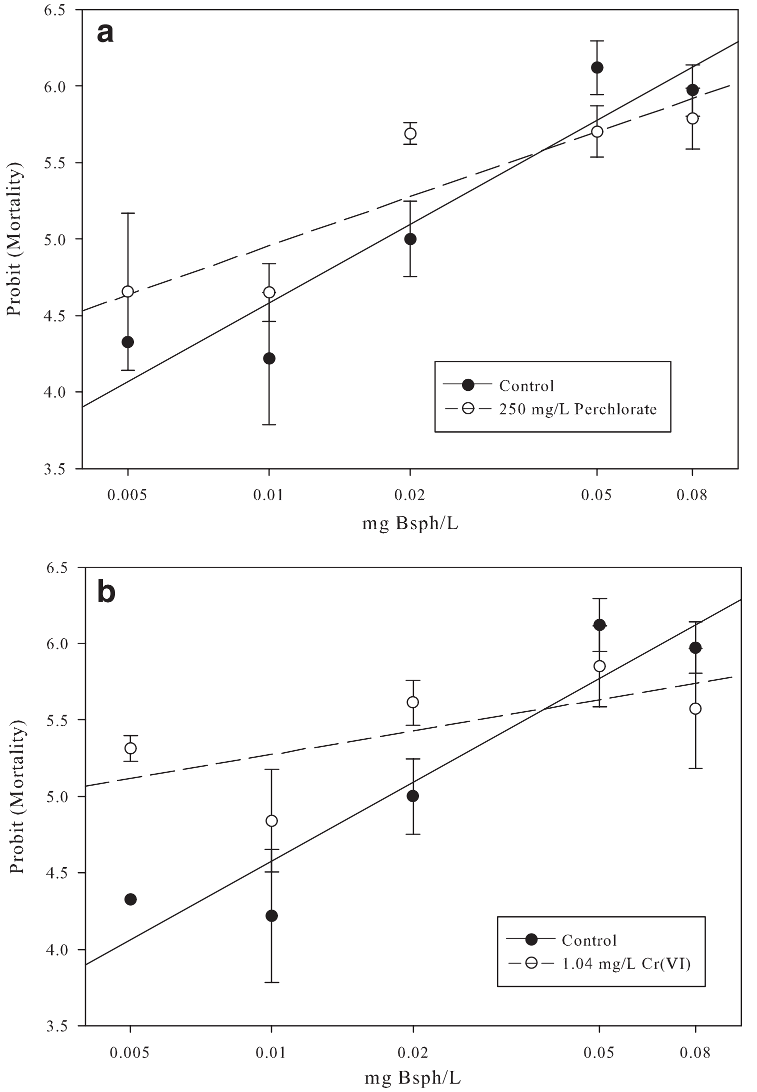

Fig. 2. Mortality of fourth instars of *Cx. quinquefasciatus* reared in water contaminated with 250 mg/liter perchlorate (a) or 1.04 mg/liter hexavalent chromium (b) and then treated with *B. sphaericus*. Dose of Bsph is plotted on a log scale. Vertical bars represent SE.

(ANCOVA: $F_{1,\ 34} = 2.84$; $P = 0.099$). However, 1.04 mg/liter chromium(VI) significantly increased Bsph efficacy (Fig. 2B) (ANCOVA: $F_{1,\ 34} = 6.24$; $P = 0.016$). Results must be interpreted with caution because of the covariate × treatment interaction (ANCOVA: $F_{34} = 4.78$ $P = 0.013$), indicating that probit lines are not parallel and the effect of the pollutant was not equal across concentrations of Bsph. Figure 2B shows that the dose response of the chromium(VI) group was fundamentally different than that of the control: the linear relationship for response of *Cx. quinquefasciatus* to Bsph is greater for the chromium treatment than for the controls. The chromium(VI)-exposed group had an LC_{50} value ≈80% less than that of the control group (Table 1). Thus, as seen with Bti, an increase in larval *Cx. quinquefasciatus* mortality would be expected with Bsph applications made in the presence of ecologically relevant levels of chromium(VI).

Discussion

It is unlikely that perchlorate at most sites will have an impact on the use of *Bacillus* larvicides to control mosquitoes. A previous study tested the effects of perchlorate on vertebrates at up to 100 mg/liter (Bernhardt et al. 2006), a concentration probably occurs at only a few sites. Toxic effects on larval mosquito growth and survival were found at 25 mg/liter perchlorate (Sorensen et al. 2006), which is beyond the upper limit of what is reported to occur in the environment, even when considering the potential for evaporative concentration (Sorensen and Trumble 2004). Therefore, it is unlikely that lower levels of perchlorate occurring in most contaminated habitats would alter the efficacy of *Bacillus* microbial control agents.

Chromium(VI), however, caused an increase in the efficacy of Bti and Bsph at ecologically relevant concentrations. A level of 1.04 mg/liter is within the range of chromium contamination found in contaminated flowing freshwater systems in the United States. Concentrations of chromium(VI) in suspended particulates, which would be available in part to mosquito larvae, can reach 2000 mg/kg in these habitats (Eisler 1986), greatly increasing the potential exposure. In such environments, mosquito control could be achieved with less product. However, the label instructions for Aquabac (Becker Microbial Products, Greely, CO), an example of a commercial Bti product, call for an application rate of ≈0.008 mg/liter, assuming a 1-m water depth. Similarly, label instructions for Vectolex (Valent Biosciences Corporation), assuming a 1-m water depth, call for 0.04 mg/liter. These representative application rates for Bti and Bsph are much higher than the LC_{50} values reported here and in other studies (Zahiri and Mulla 2003, Su and Mulla 2004, Zahiri et al. 2004), and they are designed to provide the maximum amount of control possible, even under adverse conditions or variable water depths that will likely exceed 1 m. Given these label rates of application, no change in the procedures for use of *Bacillus* microbial agents would be necessary.

Of possible concern is an increase in susceptibility of nontarget organisms to Bti or Bsph if these organisms are concurrently exposed to chromium(VI). Boisvert and Boisvert (2000) list 17 families in four orders that are negatively affected by Bti at label application rates. In the presence of chromium(VI), we would predict, based on our results here, that nontarget *Bacillus*-sensitive species may be at more of a risk from Bsph than from Bti in insecticide-treated waters contaminated with chromium (LC_{50} for Bti only changed by 21%, whereas the LC_{50} for Bsph changed by 80%). This prediction assumes that mosquito susceptibility is representative of other invertebrates; more research will be required to test this prediction.

Many factors are known to increase the effectiveness of *Bacillus* larvicides, including increased water temperature, decreased larval density, decreased sunlight, low salinity, lack of interspecific competition, shallow water, and absence of macrophytes covering the water surface (Becker et al. 1992, Charbonneau et al. 1994, Nayar et al. 1999). This study demonstrates that although perchlorate affects neither Bti nor Bsph efficacy, the presence of at least one type of metal pollution, chromium(VI), will increase the efficacy of both Bti and Bsph. Further testing will need to be done to determine whether habitat pollution with other anthropogenic contaminants will cause a similar effect.

Acknowledgments

We thank W. Carson, G. Kund, and C. Butler for critical review of this manuscript. The statistical advice and critical review of R. Redak are appreciated. Support for this study was provided through the EPA STAR program, project RD-83084101, and through the National Research Initiative of USDA–Cooperative State Research, Education and Extension Service grant 2003-35101-13630.

References Cited

Amalraj, D. D., S. S. Sahu, P. Jambulingam, P.S.B. Doss, M. Kalyanasundaram, and P. K. Das. 2000. Efficacy of aqueous suspension and granular formulations of *Bacillus thuringiensis* (Vectobac) against mosquito vectors. Acta Trop. 75: 243–246.

Becker, N., D. Petric, C. Boase, J. Lane, M. Zgomba, C. Dahl, and A. Kaiser. 2003. Mosquitoes and their control. Kluwer Academic/Plenum Publishers, New York.

Becker, N., M. Zgomba, M. Ludwig, D. Petric, and F. Rettich. 1992. Factors influencing the activity of *Bacillus thuringiensis* var. *israelensis* treatments. J. Am. Mosq. Control Assoc. 8: 285–289.

Bernhardt, R. R., F. A. von Hippel, and W. A. Cresko. 2006. Perchlorate induces hermaphroditism in threespine stickebacks. Environ. Toxicol. Chem. 25: 2087–2096.

Boisvert, M., and J. Boisvert. 2000. Effects of *Bacillus thuringiensis* var. *israelensis* on target and nontarget organisms: a review of laboratory and field experiments. Biocontrol Sci. Technol. 10: 517–561.

Canivet, V., P. Chambon, and J. Gilbert. 2001. Toxicity and bioaccumulation of arsenic and chromium in epigean and hypogean freshwater macroinvertebrates. Arch. Environ. Contam. Toxicol. 40: 345–354.

Charbonneau, C. S., R. D. Drobney, and C. F. Rabeni. 1994. Effects of *Bacillus thuringiensis* var. *israelensis* on nontarget benthic organisms in a lentic habitat and factors affecting the efficacy of the larvicide. Environ. Toxicol. Chem. 13: 267–279.

Committee to Assess the Health Implications of Perchlorate Ingestion. 2005. Health implications of perchlorate ingestion. National Research Council. National Academies Press, Washington, DC.

Dasgupta, P. K., P. K. Martinelango, W. A. Jackson, T. A. Anderson, K. Tian, R. W. Tock, and S. Rajagopalan. 2005. The origin of naturally occurring perchlorate: the role of atmospheric processes. Environ. Sci. Technol. 39: 1569–1575.

Eisler, R. 1986. Chromium hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish and Wildlife Service Biological Report 15. U.S. Fish and Wildlife Services, Washington, DC.

Elbetieha, A., and M. H. Al-Hamood. 1997. Longterm exposure of male and female mice to trivalent and hexavalent chromium compounds: effect on fertility. Toxicology 116: 19–47.

Gunasekaran, K., G. Prabakaran, and K. Balaraman. 2002. Efficacy of a floating sustained release formulation of *Bacillus thuringiensis* ssp. *israelensis* in controlling *Culex quinquefasciatus* larvae in polluted water habitats. Acta Trop. 83: 241–247.

Hogue, C. 2003. Rocket-fueled river. Chem. Eng. News 37, August 18.

Karch, S., Z. A. Manzambi, and J. J. Saluan. 1991. Field trials with Vectolex (*Bacillus sphaericus*) and Vectobac (*Bacillus thuringiensis* (H-14)) against *Anopheles gambiae* and *Culex quinquefasciatus* breeding in Zaire. J. Am. Mosq. Control Assoc. 7: 176–179.

Kotas, J., and Z. Stasicka. 2000. Chromium occurrence in the environment and methods of its speciation. Environ. Pollut. 107: 263–283.

Leslie, H. A., T. I. Pavluk, A. bij de Vaate, and M.H.S. Kraak. 1999. Triad assessment of the impact of chromium contamination on benthic macroinvertebrates in the Chusovaya River (Urals, Russia). Arch. Environ. Contam. Toxicol. 37: 182–189.

Lifsher, M. 2004. PG&E's toxic plume creeps toward LA water supply. Los Angeles Times, Los Angeles, CA. 6 March 2004.

Los Angeles Regional Water Quality Control Board. 2004. Chromium Contamination in the San Fernando Valley. (http://www.swrcb.ca.gov/rwqcb4/html/water_qty/chromium_S1.html).

Minitab, Inc. 2000. Release 13 for Windows. Minitab, Inc., State College, PA.

Nayar, J. K., J. W. Knight, A. Ali, D. B. Carlson, and P. D. O'Bryan. 1999. Laboratory evaluation of biotic and abiotic factors that may influence larvicidal activity of *Bacillus thuringiensis* serovar. *israelensis* against two Florida mosquito species. J. Am. Mosq. Cont. Assoc. 15: 32–42.

Smith, S., P. J. Peterson, and K.H.M. Kwan. 1989. Chromium accumulation, transport, and toxicity in plants. Toxicol. Environ. Chem. 24: 241–251.

Sorensen, M. A., and J. T. Trumble. 2004. Bioavailability and bioremediation of perchlorate salts, pp. 349–363. *In* S. G. Pendali [ed.], Recent research developments in environmental biology. Trivandrum, India.

Sorensen, M. A., P. D. Jensen, W. E. Walton, and J. T. Trumble. 2006. Acute and chronic activity of perchlorate and hexavalent chromium contamination on the survival and development of *Culex quinquefasciatus* Say (Diptera: Culicidae). Environ. Pollut. 144: 759–764.

Su, T., and M. S. Mulla. 2004. Documentation of high-level *Bacillus sphaericus* 2362 resistance in field populations of *Culex quinquefasciatus* breeding in polluted water. J. Am. Mosq. Control Assoc. 20: 405–411.

Trumble, J. T., and P. D. Jensen. 2004. Ovipositional response, developmental effects, and toxicity of hexavalent chromium to Megaselia scalaris, a terrestrial detritivore. Arch. Environ. Contam. Toxicol. 46: 372–376.

Urbansky, E. T. 1998. Perchlorate chemistry: implications for analysis and remediation. Bioremediation J. 2: 81–95.

[U.S. EPA] U.S. Environmental Protection Agency. 2002. Perchlorate environmental contamination: toxicological review and risk characterization (2002 external review draft). Office of Research and Development, National Center for Environmental Assessment, Washington office, Washington, DC.

[U.S. EPA] U.S. Environmental Protection Agency. 2005a. National perchlorate detections as of September 23, 2004. (http://www.epa.gov/fedfac/documents/perchlorate_map/nationalmap.htm).

[US EPA] U.S. Environmental Protection Agency. 2005b. Known perchlorate releases in the U.S.-March 25, 2005. (http://www.epa.gov/fedfac/documents/detection_with_dates_03_25_05.xls).

Vuori, K. M., and J. Kukkonen. 1996. Metal concentrations in *Hydropsyche pellucida* larvae (Trichoptera, Hydropsychidae) in relation to the anal papillae abnormalities and age of exocuticle. Water Res. 30: 2265–2272.

Wolff, J. 1998. Perchlorate and the thyroid gland. Pharmacol. Rev. 50: 89–106.

Zahiri, N. S., B. A. Federici, and M. S. Mulla. 2004. Laboratory and simulated field evaluation of a new recombinant of *Bacillus thuringiensis* spp. *israelensis* and *Bacillus sphaericus* against *Culex* mosquito larvae (Diptera: Culicidae). J. Med. Entomol. 41: 423–429.

Zahiri, N. S., and M. S. Mulla. 2003. Susceptibility profile of *Culex quinquefasciatus* (Diptera: Culicidae) to *Bacillus sphaericus* on selection with rotation and mixture of *B. sphaericus* and *B. thuringiensis israelensis.* J. Med. Entomol. 40: 672–677.

Zhang, J., and X. Li. 1987. Chromium pollution of soil and water in Jinzhou. J. Chin. Prevent. Med. 21: 262–264.

Received 4 January 2007; accepted 31 May 2007.