Arch. Environ. Contam. Toxicol. 46, 372–376 (2004)
DOI: 10.1007/s00244-003-3007-8

ARCHIVES OF
Environmental
Contamination
and Toxicology

Ovipositional Response, Developmental Effects and Toxicity of Hexavalent Chromium to *Megaselia scalaris,* a Terrestrial Detritivore

John T. Trumble, Peter D. Jensen

Department of Entomology, University of California, Riverside, California 92521, USA

Received: 29 January 2003/Accepted: 1 July 2003

Abstract. The effects of hexavalent chromium (Cr VI) on ovipositional response, development, and survival of a common terrestrial detritivore, *Megaselia scalaris* (Diptera: Phoridae), were assessed in the laboratory. Ovipositing females did not discriminate between substrates containing 0, 50, 500, or 1000 μg/g, indicating a lack of avoidance behavior. Eggs placed on artificial diets containing up to 1000 μg/g either did not absorb Cr VI or were unaffected as measured by eclosion rates. However, development and survival of larvae were significantly reduced at the higher concentrations tested. Concentrations of 500 μg/g in their food increased larval development times by nearly 65%. At 1000 μg/g, larval developmental times doubled. The time required from onset to completion of pupariation was not significantly different regardless of Cr VI concentration. Although males eclosed before females, there was no significant difference between the sexes in the time required for adult eclosion. In addition, there were no significant differences in the percentage of males and females emerging from any of the treatments. At concentrations of 500 or 1000 μg/g, Cr VI decreased larval survival. Survival was reduced by 44.3% at 500 μg/g as compared with the controls. There was no additional mortality from the onset of the puparial stage to adult eclosion for larvae fed diets containing 500 μg/g Cr VI. At 1000 μg/g Cr VI, larval survival decreased by 86.6%. An additional 7.4% mortality was recorded in the puparial stage, for a decrease in total survival (larval plus puparial stages) of 94%. Thus, nearly all of the observed mortality occurred during the larval stage rather than the puparial stage. The population level implications of lack of avoidance of contaminated food and the effects of increased developmental times and reduced survivorship are discussed.

Water and soil contamination with chromium is a significant worldwide problem (World Bank 2002; http://www.worldbank.org/nipr/polmod.htm). Contamination is associated with a variety of industrial processes, including chrome plating baths, chrome colors and dyes, cement manufacturing, leather tanning agents, wood preservatives, anticorrosive agents, welding fumes, lubricating oils and greases, many cleaning materials, and textile production (Kimbrough *et al.* 1999; Kotas and Stasicka 2000). Chromium occurs in several oxidation states with the two major oxidation states, trivalent chromium (Cr III) and hexavalent chromium (Cr VI), behaving very differently in the environment. Chromium III, which is important nutritionally for animals, can form insoluble precipitates and behaves as a hard Lewis acid (Wang *et al.* 1997). Cr VI is highly soluble in water and toxic to both plants and animals (Zhang and Li 1987; Smith *et al.* 1989; Elbetieha and Al-Hamood 1997). Public concern is related mainly to Cr VI, since it has been found dissolved in drinking water and toxicity is associated with the oxidation of intracellular compounds (Costa 1997).

Hexavalent chromium is known to accumulate in plants. Concentrations in an aquatic plant (*Nelumbo nucifera*) reached 2,040 μg/g dry weight (dw) in leaves and 3,170 μg/g dw in the roots (Vajpayee *et al.* 1999). Chromium levels (specific form not reported) in seeds of *Euryale ferox* (an edible aquatic plant) exceeded 650 μg/g, bioaccumulating from sediment levels of approximately 120 μg/g (Rai *et al.* 2002). Even short exposures allowed substantial accumulations in plants. Alligator weed, *Alternanthera philoxeroides,* exposed to 10 mg/kg Cr03 for 3 weeks accumulated 214 mg/kg fresh weight (fw) in the roots (Naqvi and Rizvi 2000). The concentration in cauliflower roots exceeded 500 mg/kg fw when exposed for one week to 1 mg/L Cr VI (19.2 1M Cr) (Zayed *et al.* 1998). Lettuce roots also accumulate Cr VI (Peijnenburg *et al.* 2000), but transfer of Cr VI from roots to leaves in several vegetable species appears limited (Zayed *et al.* 1998; Peijnenburg *et al.* 2000). In other studies, some plants such as mosses concentrated Cr from water even when the plant was no longer living (Gstoettner and Fisher 1997). Thus, there is ample evidence for availability of Cr-contaminated plant material in many ecosystems.

Most studies on Cr toxicity have been conducted on aquatic organisms such as brine shrimp (Hadjispyrou *et al.* 2001), *Daphnia* species (Kungolos and Aoyama 1993), crabs (*Portuns pelagicus,* Al-Mohanna and Subramanyam 2001), and crayfish (*Procambarus clarkii,* Bollinger et al. 1997). We found few studies that examined the effects of Cr VI on insects, and these investigations focused on an aquatic species. Leslie *et al.* (1999) studied caddisflies (*Hydropsyche pellucidula:* Trichoptera) exposed to chromium contamination (including

Correspondence to: J. T. Trumble; *email:* john@ucr.edu

both Cr VI and Cr III) of 922 ± 102 mg/L. They found the anal papillae of immatures were damaged when exposed to high concentrations of chromium. The anal papillae are ion-exchange organs that are responsible for taking up ions from the water, ensuring the hypertonicity of the insect's body fluids with respect to the surrounding aquatic environment. Papillae damage is typically accompanied by reduced fitness of affected individuals (Vuori 1994). Leslie et al. (1999) also found evidence of bioaccumulation of Cr and physical abnormalities in many surviving caddisfly larvae. Thus, information on the effects of Cr VI on insects is quite limited, and data on terrestrial insect herbivores and detritivores appear to be entirely lacking. For our study, we examined the effects of Cr VI on a terrestrial insect in an artificial diet in order to eliminate potentially confounding effects of plant defensive chemicals, pollutant mixtures, and variable nutrition. We hypothesized that despite the variation in routes of exposure between arthropods in terrestrial and aquatic systems, ingestion of Cr VI by a terrestrial arthropod would also have deleterious effects.

Materials and Methods

Megaselia scalaris

(Loew.) (Diptera: Phoridae), a small yellowish-brown phorid of nearly cosmopolitan distribution, was selected as a test organism. The species has been found throughout North America and in Asia, Africa, and Europe (Mainx 1964; Robinson 1975). The females copulate shortly after emerging as adults and oviposit soon thereafter (Mainx 1964). Eggs are laid directly on the food, and larvae are capable of limited movement within the host material. Larvae of this species have been reported developing on a wide variety of host materials including dead mammals and insects, and a broad selection of decomposing plant material (Trumble and Pienkowski 1979). Thus, this species functions as a detritivore and is likely to feed on decaying organisms (plants or animals) contaminated with many different pollutants, including Cr VI. After completion of the larval stage, the larvae pupariate (essentially pupate within the last larval integument). At 27°C, larval development averages about 7 days, and the puparial stage averages approximately 10 days; development is not affected by short (LD: 12–12) or long day photoperiods (LD: 16–8) (Trumble and Pienkowski 1979).

A laboratory colony was established in 2001 from adult flies found feeding on an alfalfa-based diet (Mandeville *et al.* 1988) used for another insect species. The flies were reared at a constant 26°C with a photoperiod of 16:8 L:D. For all tests, age of the eggs was synchronized by allowing groups of approximately 25 adult females from the colony to oviposit on Fisher's *Drosophila* diet (Fisher Scientific, Pittsburgh, PA, USA) in Petri dishes for 2 h.

Hexavalent chromium was dissolved in HPLC grade water and used to make *Drosophila* diets (Instant Drosophila Medium, Fisher Scientific, Chicago, IL) containing 0 (control) to 1000 μg/g Cr VI. These levels are within the ranges found occurring in plants at contaminated sites (Vajpayee *et al.* 1999). The blue diet provided excellent contrast with the white eggs, white larvae, and yellow-brown puparia, greatly facilitating counts. To achieve the desired concentrations of Cr VI, A.C.S-certified chromium trioxide (Fisher Scientific) was serially diluted and then used to hydrate the diet. All bioassays were maintained at 28 ± 2°C, ca. 75% RH, and a 14:10 (L:D) photoperiod with fluorescent lighting. Tests were conducted for ovipositional preference, effects on egg hatch, development rate, and survival.

Oviposition Studies

To measure the potential for ovipositional preference or avoidance of contaminated food, were prepared diets using 0 (control), 50, 500, and 1000 μg/g of Cr VI. Diets initially were dispensed into the bases of Petri dishes to a depth of 1.2 cm. A #9 cork borer was then used to cut cylinders of diet (1.2 cm depth × 1.2 cm diameter) from diets with each Cr VI concentration. These cylinders provided uniform shapes and quantities of diet for oviposition. The diet cylinders were arranged in groups of four in larger Petri dishes (2.2 cm depth × 7.5 cm diameter), with pairs of controls alternating with pairs of cylinders containing a single concentration. Each cylinder was separated by approximately 4 cm from all other cylinders. Approximately 10 pairs of flies were introduced into each dish and allowed to oviposit for 1.5 days. Each concentration was replicated 10 times, requiring ca. 600 adult flies for the test. At the end of the oviposition period, the numbers of eggs on treatment and control cylinders were recorded for each dish by using a microscope. All eggs were laid in direct contact with a diet cylinder. None of these eggs were used in any other studies. Oviposition preference was analyzed with the Wilcoxon Signed Rank nonparametric procedure for paired comparisons (Stat View 2001; Tallamy *et al.* 1997; Vickerman *et al.* 2002).

Because of a known potential for insect eggs to absorb liquid from the surface of the diet (Chapman 1975) and thereby possibly acquire Cr VI, an additional analysis was conducted to determine whether numbers of non-hatching eggs differed with Cr VI concentration. In order to get comparable replicates of eggs from a general source of flies, an oviposition substrate was placed in the colony and adults were allowed to oviposit for 2 h. These eggs were then serially transferred to the surface of *Drosophila* diets containing concentrations of 0, 0.05, 0.5, 50, 500, and 1000 μg/g Cr VI. The numbers of eggs eclosed after 12 h were assessed for eight replicates of 20 eggs per concentration. These rates were compared against the controls by one way ANOVA ($P = 0.05$) (Stat View 2001).

Development and Survival Studies

Hexavalent chromium-contaminated *Drosophila* diet was prepared as described earlier in concentrations of 0, 0.05, 0.5, 50, 500, and 1000 μg/g. These diets were dispensed into one side of 75-ml clear plastic, divided Petri dishes. The larvae either move to the surface or left the food to pupariate (Trumble and Pienkowski 1979), and the diet-free side provided a preferred site for pupariation in our test. Each Petri dish received 20 eggs. Additionally, potential confounding effects of slowed eclosion or egg mortality were eliminated by removing any eggs that had not hatched within 12 h (mean < 10%). Eight replicate Petri dishes containing 20 eggs each were tested for each concentration, for a total of 960 eggs. The dishes were examined daily, and date and numbers of puparia were recorded. The date of adult emergence and sex of emerging adults were also recorded daily for each dish for 36 days, at which time all individuals had completed development to the adult stage or had died. Diets remained acceptable for the length of the experiment and were not changed. Surviving larvae were never observed to feed on dead larvae. This procedure allowed documentation of the larval development period, the duration of puparial development, and the total (larval and puparial) developmental time. All development and survival data were analyzed by one-way Analysis of Variance (ANOVA, $P = 0.05$ level) (Stat View 2001). Percentage data were transformed with an arcsine square root transformation prior to analysis. As appropriate, ANOVA was followed by a post hoc analysis with Tukey's HSD to determine developmental or survival differences between individual concentrations.

Results

Oviposition Preference Study

Female *Megaselia scalaris* did not show any preference or avoidance of food contaminated with CR VI at concentrations up to 1000 μg/g (*P* values all ≥ 0.14, overall mean = 64% on control). No significant differences in eclosion rate were detected for eggs oviposited on control diets as compared with any of the concentrations tested (mean range 15.9–18.9; $P > 0.15$; ANOVA). Thus, either the eggs did not absorb appreciable concentrations of Cr VI or the Cr VI did not have measurable effects on this life stage. Regardless, eggs laid on Cr VI-contaminated food could be expected to eclose in normal numbers, at least up to concentrations of 1000 μg/g.

Development and Survival Studies

Larval development times increased significantly at the highest concentrations of Cr VI (Fig. 1). Concentrations of 500 μg/g in their food increased larval development times by nearly 65%. At 1000 μg/g, larval developmental times doubled.

Development to the adult stage was similarly affected (Table 1). Concentrations at 500 μg/g and above significantly increased the time required to reach adult eclosion for both males and females. However, this increase could be attributed to effects occurring in the larval stage because time spent in the puparial stage was not significantly different between treatment concentrations (Table 1). There was no significant difference between the sexes in the time required for adult eclosion ($P > 0.32$; ANOVA). In addition, there were no significant differences in the percentage of females emerging from any of the treatments (overall mean = 50.2%; $P > 0.95$; ANOVA).

At concentrations of 500 or 1000 μg/g, Cr VI decreased both larval survival and survival to the adult stage (Fig. 2). Larval survival was reduced by 44.3% at 500 μg/g as compared with the controls. There was no additional mortality from the onset of the puparial stage to adult eclosion. At 1000μg/g, larval survival decreased by 86.6%. An additional 6.4% mortality was recorded in the puparial stage, for a decrease in total survival (larval plus puparial stages) of 94%. Thus, nearly all of the observed mortality occurred during the larval stage rather than the puparial stage.

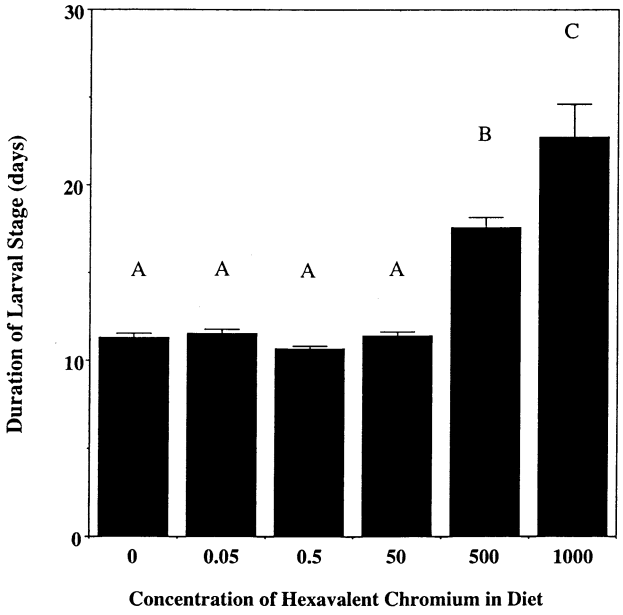

Fig. 1. Effect of various hexavalent chromium concentrations on time to adult eclosion for *Megaselia scalaris.* Bars (means ± SE) with different letters are significantly different at the $P < 0.05$ level (ANOVA, Tukey's HSD test)

Table 1. Development times for puparia, males and females of *Megaselia scalaris* feeding on diets containing hexavalent chromium[a]

Concentration of Cr VI (μg/g)	Mean days to adult eclosion		Mean days in the puparial stage
	Males	Females	
0.00	20.2 a	21.1 a	9.7 a
0.05	19.2 a	20.4 a	9.6 a
0.50	19.8 a	20.7 a	10.8 a
50.00	19.7 a	21.4 a	9.3 a
500.00	26.4 b	27.0 b	9.4 a
1000.00	27.0 b	29.2 b	7.2 a

[a] Numbers within columns followed by the same letter are not significantly different at the $P < 0.05$ level, ANOVA.

Discussion

Because *M. scalaris* larvae are not capable of substantial movement, immatures are essentially restricted to the food selection made by the adult female at the time of oviposition. Thus, the lack of avoidance behavior by adults will result in exposure of offspring to Cr VI in contaminated sites. Because of a long-standing focus on toxicological effects of pollutants, only one other study is available on behavioral effects of chromium on insects. Lefcort *et al.* (2000) determined that four caddisfly species from four different genera did not lose antipredatory defensive behaviors when exposed to a mixture of Cr III and Cr VI. Nonetheless, our study suggests that additional emphasis should be placed on behavioral responses of insects to generate a more complete description of the effects of chromium and other metals on ecosystem ecology.

Relatively few studies are available on effects of toxicants on population growth factors; Stark and Banks (2003) reported that 95% of the literature they reviewed used mortality and median lethal dose/concentration as a toxicological endpoint. The importance of Cr VI on developmental rates underscores this point. The observed increases in development time at the higher concentrations would decrease the intrinsic rate of population increase and expose the remaining larvae to additional mortality from environmental factors or predators. Such increases in time for development would likely impact population fitness in many natural systems because the availability of food sources for the larvae are often temporary (Trumble and Pienkowski 1979), and extending larval developmental times could exceed the suitability of the food supply. Measurement of potential effects on adults was beyond the scope of this

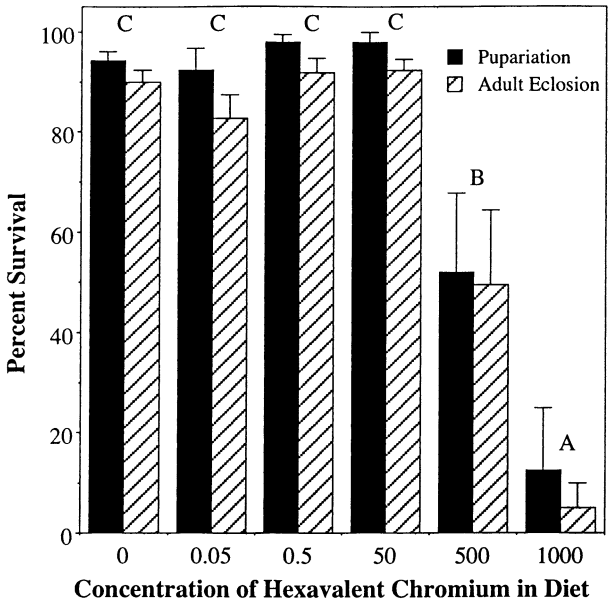

Fig. 2. Survival from egg hatch until pupariation (solid bars) and from egg hatch until adult eclosion (hatched bars). Bars (means ± SE) with different letters are significantly different at the $P < 0.05$ level (ANOVA, Tukey's HSD test) for survival within a category

paper. However, any Cr VI-induced changes in fecundity, adult longevity, or altered predation patterns would likely efffect population fitness.

We chose to work with the egg, larval, and puparial stages of *M. scalaris* to measure effects of Cr VI across the entire immature lifespan. Studies of acute toxicity are undeniably useful, but can be misleading. For example, Canivet *et al.* (2001) tested the toxicity of Cr VI to larvae of two aquatic insect species (*Heptagenia* sp. and *Hydropsiche* sp.), three aquatic crustaceans (*Gammarus* sp., *Niphargus* sp., and *Asellus* sp.), and a snail (*Physa* sp.). LC_{50}s were determined over 96- and 240-h periods; toxicity increased with longer exposure times. However, the increased toxicity was not equivalent across species. The relative toxicity at 240 h as compared with 96 h increased twofold for snails, while chromium toxicity to various insect and crustacean species ranged from 6- to 26-fold. While the differences between dermal exposure to a contaminant (as seen for aquatic invertebrates) versus oral exposure to a contaminant (as occurs with terrestrial hervivores or detritivores) preclude direct comparisons of LC_{50} values for Cr toxicity, the general pattern of more accurate estimates of population level effects with longer exposures is evident. In our study, concentrations of Cr VI as low as 500 μg/g caused significant reductions in survival. However, nearly all the mortality occurred in the larval stage as opposed to the puparial stage, and no sex-linked effects were documented, so future investigations with Cr VI with *M. scalaris* could reliably focus on just the larval stage as a reasonable measure of potential adverse effects.

Little is known regarding the effects of various forms of chromium on insects. Such data are important because: 1) the valent states of metals and metalloids have been shown to have very different impacts on insects (Trumble *et al.* 1998; Vickerman and Trumble 1999) and 2) insects occupy broad niche dimensions in nearly every terrestrial and freshwater ecosystem and are, therefore, responsible for a significant amount of energy transfer from primary producers to carnivores (Price *et al.* 1974). Although the present study examined the effects of Cr VI on an insect on an artificial diet in order to eliminate confounding effects of plant defensive chemicals, pollutant mixtures, and variable nutrition, future investigations that incorporate these variables will be required before accurate predictions can be made regarding the effects of Cr VI on ecosystem function.

Acknowledgments. We appreciate the identification of *M. scalaris* by B. Brown, Natural History Museum of Los Angeles County. The laboratory assistance of L. Johnson and M. Arias is also appreciated. We thank W. Carson, G. Kund, and D. Liu for their critical reviews of this manuscript. The UC Toxic Substances Research and Teaching Program, EPA grant 83084101, and UCR Dean's Office Fellowship provided initial support for this project.

References

Al-Mohanna SY, Subramanyam MNV (2001) Flux of heavy metal accumulation in various organs of the intertidal marine blue crab, *Portunus pelagicus* (L.) from the Kuwait coast after the Gulf War. Environ Int 27:321–326

Bollinger JE, Bundy K, Bitner Anderson M, Millet L, Preslan JE, Jolibois L, Chen H-L, Kamath B, George WJ (1997) Bioaccumulation of chromium in red swamp crayfish. J Hazard Mater 54: 1–13

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

Chapman RF (1975) The insects, structure and function. American Elsevier, New York

Costa M (1997) Toxicity and carcinogenicity of Cr(VI) in animal models and humans. Crit Rev Toxicol 27:431–442

Elbetieha A, Al-Hamood MH (1997) Long-term exposure of male and female mice to trivalent and hexavalent chromium compounds: effect on fertility. Toxicology 116:19–47

Gstoettner EM, Fisher NS (1997) Accumulation of cadmium, chromium and zinc by the moss *Sphagnum papillosum,* Lindle. Water Air Soil Pollut 93:321–330

Hadjispyrou S, Kungolos A, Anagnostopoulos A (2001) Toxicity, bioaccumulation, and interactive effects of organotin, cadmium, and chromium on *Artemia franciscana.* Ecotoxicol Environ Saf 49:179–186

Kimbrough DE, Cohen Y, Winer AM, Creelman L, Mabuni C (1999) A critical assessment of chromium in the environment. Crit Rev Environ Sci Technol 29:1–46

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

Kungolos A, Aoyama I (1993) Interaction effect, food effect and bioaccumulation of cadmium and chromium for the system *Daphnia magna – Chlorella ellipsoidea.* Environ Toxicol Water Qual 8:351–369

Lefcort H, Ammann E, Eiger SM (2000) Antipredatory behavior as an index of heavy metal pollution? A test using snails and caddis flies. Arch Environ Contam Toxicol 38:311–316

Leslie HA, Pavluk TI, Vaate A, Kraak MHS (1999) Triad assessment of the impact of chromium contamination on benthic macroinver-

tebrates in the Chusovaya River. Arch Environ Contam Toxicol 37:182–189

Mainx F (1964) The genetics of *Megasilia scalaris* Loew (Phoridae): a new new type of sex determination in Diptera. Am Nat 98:415–430

Mandeville JD, Mullens BA, Meyer JA (1988) Rearing and host age suitability of *Fannia canicularis* (L.) (Diptera: Muscidae) for parasitization by *Muscidifurax zaraptor* Kogan and Legner (Hymenoptera:Pteromalidae). Can Entomol 120:153–159

Naqvi SM, Rizvi SA (2000) Accumulation of chromium and copper in three different soils and bioaccumulation in an aquatic plant *Alternanthera philoxeroides.* Bull Environ Contam Toxicol 65: 55–61

Peijnenburg W, Baerselman R, de Groot A, Jager T, Leenders D, Posthuma L, Van Veen R (2000) Quantification of metal bioavailability for lettuce (*Lactuca sativa* L.) in field soils. Arch Environ Contam Toxicol 39:420–430

Price PW, Rathcke BJ, Gentry DA (1974) Lead in terrestrial arthropods: evidence for biological concentration. Environ Entomol 3:370–372

Rai UN, Tripathi RD, Vajpayee P, Jha V, Ali MB (2002) Bioaccumulation of toxic metals (Cr, Cd, Pb, and Cu) by seeds of *Euryale ferox* Salisb. (Makhana). Chemosphere 46:267–272

Robinson, WH (1975) *Megasilia scalaris* (Diptera: Phoridae) associated with laboratory cockroach colonies. Proc Entomol Soc Wash 77:384–390

Smith S, Peterson PJ, Kwan KHM (1989) Chromium accumulation, transport and toxicity in plants. Toxicol Environ Chem 24:241–251

Stark JD, Banks JE (2003) Population-level effects of pesticides and other toxicants on arthropods. Annu Rev Entomol 48:505–519

StatView (2001) Version 5.0.1. SAS Institute, Cary, NC

Tallamy DW, Stull J, Ehresman NP, Gorski PM, Mason CE (1997) Cucurbitacins as feeding and oviposition deterrents to insects. Environ Entomol 26:678–683

Trumble JT, Pienkowski RL (1979) Development and survival of *Megaselia scalaris* (Diptera: Phoridae) at selected temperatures and photoperiods. Proc Entomol Soc Wash 81:207–210

Trumble JT, Kund GS, White KK (1998) Influence of form and quantity of selenium on the development and survival of an insect herbivore. Environ Pollut 101:175–182

Vajpayee P, Sharma SC, Tripathi RD, Rai UN, Yunus M (1999) Bioaccumulation of chromium and toxicity to photosynthetic pigments, nitrate reductase activity and protein content of *Nelumbo nucifera* Gaertn. Chemosphere 39:2159–2169

Vickerman DB, Trumble JT (1999) Feeding preferences of *Spodoptera exigua* in response to form and concentration of selenium. Arch Insect Biochem Physiol 42:64–73

Vickerman DB, Shannon MC, Bañuelos GS, Grieve CM, Trumble JT (2002) Evaluation of *Atriplex* lines for selenium accumulation, salt tolerance and suitability for a key agricultural insect pest. Environ Pollut 120:463–473

Vuori KM (1994) Rapid behavioural and morphological responses of Hydropsychid larvae (Trichoptera; Hydropsychidae) to sublethal cadmium exposure. Environ Pollut 84:291–299

Wang WX, Griscom SB, Fisher NS (1997) Bioavailability of Cr(III) and Cr(VI) to marine mussels from solute and particulate pathways. Environ Sci Technol 31:603–611

World Bank (2002) The industrial pollution projection system. (http://www.worldbank.org/nipr/polmod.htm)

Zayed A, Lytle CM, Qian J-H, Terry N (1998) Chromium accumulation, translocation and chemical speciation in vegetable crops. Planta 206:293–299

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