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Acute and chronic activity of perchlorate and hexavalent chromium contamination on the survival and development of *Culex quinquefasciatus* Say (Diptera: Culicidae)

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While pollution with hexavalent chromium may adversely affect Culex quinquefasciatus larvae, levels of perchlorate currently in the environment will not impact these insects.

Abstract

Effects of water contamination with perchlorate and hexavalent chromium [Cr (VI)] on the mosquito *Culex quinquefasciatus* were assessed. The chronic (10-day) LC_{50}s values for perchlorate and chromium were 74 ± 8.0 mg/L and 0.41 ± 0.15 mg/L, respectively. Relative Growth Index, a measure of growth and mortality rates in a population, was significantly reduced within 5 days for levels of perchlorate as low as 25 mg/L and for levels of chromium as low as 0.16 mg/L. Neither compound altered wing length of surviving adults. In combination, contaminants were synergistic, causing 14% more mortality than predicted. Acute (24-h) LC_{50} values for perchlorate and Cr (VI) were $17{,}000 \pm 3200$ and 38 ± 1.3 mg/L, respectively. Effects on mosquito larvae in contaminated environments are likely to be observed for Cr (VI) but not for perchlorate, which generally does not occur at levels as high as those shown here to affect larval mosquitoes.

Key words: Joint toxicity; Subchronic toxicity; Mosquito; Insect; Pollution

Anthropogenic pollutants of many types have become pervasive in environments throughout the world, yet the impacts that these contaminants have on species and ecosystems are not well understood. Some pollutants have been accumulating for years, but are only now of environmental concern, due to improved detection abilities. Most reports describe the effects of individual pollutants, with relatively few studies including the biological or ecological effects of mixtures of contaminants (Yang, 1994). In addition, Forbes and Calow (1999) and Stark and Banks (2003) note that most studies of toxicants used mortality and median lethal dose/concentration as a toxicological endpoint. These authors suggest that measures including both lethal and sublethal effects, such as the rate of population growth and sex ratios of offspring, would provide more accurate assessments of the impact of toxicants. Thus, in order to determine if patterns of responses exist in ecosystems, or even among species, more studies are needed that examine the effects of mixtures on the fitness of organisms.

Perchlorate (ClO_4^-) is a persistent anthropogenic pollutant. This chemical is manufactured for use as an oxidant in rocket fuels, roadside flares, airbags, fireworks, and other combustibles. In addition, some perchlorate may be produced by atmospheric processes (Dasgupta et al., 2005). Drinking or irrigation water in over half of the states in the USA has been found to be contaminated (US EPA, 2005), and 44 states have sites where perchlorate was manufactured or used at one time (US EPA, 2002). In California, more than four hundred wells and drinking water sources contain perchlorate above the 0.1 μg/L detection limit, and these are extensively used for irrigation, which makes the contaminant more bioavailable over large areas. California contains a number of hotspots for

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perchlorate contamination, particularly Los Angeles, San Bernardino, and Riverside counties. In Nevada, the now defunct Kerr-McGee Plant for rocket fuel has been responsible for up to 455 kg of perchlorate leaching daily into the Colorado River at Lake Mead (Hogue, 2003).

Perchlorate has been shown to have adverse effects on metabolism and development in mammals, amphibians, and lampreys (Goleman et al., 2002a,b), and has been found in the tissues of rodents, fish, frogs, and aquatic insects at a contaminated site (Smith et al., 2001). However, the scientific literature contains no studies of the effects of perchlorate on any insect. In mammals, perchlorate interrupts metabolism by competitively inhibiting iodine uptake by the sodium iodide symporter (NIS) into the thyroid gland (Wolff, 1998). Although insects are known to have a protein with an extensive homology to the human NIS (Benvenga et al., 1999), the role of iodine in insect metabolism is not known. For an overview of the widespread occurrence of perchlorate in the environment, see Sorensen and Trumble (2004).

Water and soil contamination with chromium are also significant worldwide problems (World Bank, 2002). Chromium has been extensively used in many industrial processes, including chrome colors and dyes, cement manufacturing, and wood preservatives. Like perchlorate, chromium has applications in the electroplating and leather tanning industries (Kotas and Stasicka, 2000). In the San Fernando Valley of Southern California, the US EPA's monitoring wells have detected concentrations as high as 1.0 mg/L (Los Angeles Regional Water Quality Control Board, 2004) and a large plume measuring more than 12 mg/L 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. Thus, the distribution of Cr (VI) contamination is such that large areas are affected, including considerable overlap with perchlorate occurrence (US EPA, 1998).

In the environment, chromium is most often found as chromium (III) or chromium (VI). Though chromium (III) is important nutritionally for animals, chromium (VI) has been found to be toxic to both animals and plants (Zhang and Li, 1987; Smith et al., 1989; Elbetieha and Al-Hamood, 1997). There are relatively few studies that have examined the effects of Cr (VI) on insects, and these focused on aquatic species in running water (Vuori and Kukkonen, 1996; Leslie et al., 1999; Canivet et al., 2001) and on a terrestrial detritivore species (Trumble and Jensen, 2004). No research has been published that investigates the interaction of perchlorate and Cr (VI) on any organism. The purpose of this study was to evaluate the risk perchlorate and hexavalent chromium, alone and in combination, may pose to aquatic insects, using *Culex quinquefasciatus* as a model organism.

1. Methods and materials

C. quinquefasciatus Say is important both as a widespread and abundant lower-trophic level member of many ecosystems and as a vector of human encephalitis viruses. Thus, changes to *C. quinquefasciatus* populations affect both ecosystem function and human health. Larvae typically take between one and two weeks to complete development to the adult stage, a short life cycle that makes *C. quinquefasciatus* advantageous for rapidly completing developmental as well as toxicological studies. Although *C. quinquefasciatus* is native to North America, the species has been introduced throughout the tropics and warm temperate regions of the world, where it utilizes birds and mammals as bloodmeal hosts. This mosquito is commonly found in urban, suburban, and rural areas, and breeds in water moderately to severely polluted with organic matter (Savage and Miller, 1995).

A *C. quinquefasciatus* colony was maintained in a laboratory at the University of California, Riverside, CA. Eggs were collected as needed and hatched in a shallow pan ($30 \times 20 \times 5$ cm) containing HPLC grade water (Milli-Q Water System, Millipore Corporation, Bedford, MA). After 3 days, larvae were transferred to bioassay containers 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. 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, LLC, Aurora, OH) re-hydrated with 50 ml of water to approximately 4 g powdered food. In all trials, each treatment was replicated four times (one jar with 20 larvae is considered a replicate), and at least three concentrations plus a control were tested for each contaminant (perchlorate or Cr (VI)). All controls consisted of bioassay jars stocked as previously described but without contaminant added. Bioassay jars were maintained in an incubation chamber at 28 ± 1 °C, 75% RH with a photoperiod of L:D 14:10.

Perchlorate was added to bioassay containers as ammonium perchlorate (CAS# 7790-98-9, NH_4ClO_4, 99.999%, Aldrich Chemicals, Milwaukee, WI). Chromium 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 the perchlorate anion (ClO_4^-). Chromium trioxide dissolved in water will form chromic acid (H_2CrO_4), but may also undergo a number of other reactions, including complexing with dissolved organic and inorganic molecules. Chromium is therefore reported here as concentration of elemental chromium (Cr).

Perchlorate treatments contained both ammonium (NH_4^+) and ammonia (NH_3) in solution. Ammonia concentrations in our treatments ranged from 0.02 to 17 mg/L. Dissolved ammonia can be toxic to aquatic life, however, *Culex* mosquitoes are known to breed and develop exceptionally large populations in dairy wastewater (Mulla and Darwazeh, 1988), where ammonia concentrations reach up to 700 mg/L (Bays, 2002; http://www.cmer.wsu.edu/Summer/bays.pdf). Thus, in our experiments, ammonia is not likely to be an important mortality factor.

1.1. Chronic toxicity

Chronic toxicity trials were conducted in two experiments. The first set of experiments tested perchlorate concentrations of 8.5, 21, 42, and 63 mg/L and Cr (VI) concentrations of 0.10, 0.16, 0.21, and 0.26 mg/L. The second set of experiments tested perchlorate concentrations of 106, 127, 148, and 169 mg/L and Cr (VI) concentrations of 0.31, 0.36, 0.42, and 0.47 mg/L. Preliminary data (Sorensen unpublished) not reported here indicate that the ranges of concentrations evaluated in these experiments encompass the LC_{50} values for perchlorate and Cr (VI). Numbers of surviving larvae and their developmental stages were recorded daily. Chronic experiments were terminated when mortality in control jars reached 80%, after approximately 10–14 days. Emerging adult mosquitoes were removed daily and their sex recorded. Adults were collected, frozen and their wing lengths measured (length from axial incision to wing tip, not including scales). Mosquito wing length correlates with body weight and has been used previously as a fitness correlate (Armbruster and Hutchinson, 2002).

Mortality was examined using log-dose probit analysis (Minitab, 2000). The Growth Index (GI) and Relative Growth Index (RGI), as described by Zhang et al. (1993), are quantitative measures that incorporate both mortality and development of a group of individual organisms. GI is calculated as:

$$\mathrm{GI} = \frac{\sum_{i=1}^{i_{\max}} [n_{(i)} \times i] + \sum_{i=1}^{i_{\max}} [n_{(i)} \times (i-1)]}{\mathrm{N} \times i_{\max}}$$

where i = the instar of the insect, $i_{max} = 6$, the highest attainable instar, n_i = the number of individuals at instar i, and N = the total starting number of individuals in a group. RGI was determined as RGI = (GI of the test group)/(GI of the control group). GI of the control group is a constant determined by the average GI of the control treatment on the last day of the experiment. If RGI values do not differ between concentrations of the same test material, then the combinations would not be considered growth inhibiting (Zhang et al., 1993). Relative Growth Index, wing length, and sex of adults were compared using ANOVA followed by Tukey's HSD post-hoc test as appropriate (Statview, 2001).

1.2. Acute toxicity

Following hatching, *C. quinquefasciatus* larvae were transferred to jars containing 90 ml of distilled water and fed as described previously. Jars contained 20 larvae, with four replicates for each concentration. When larvae reached the fourth instar, 10 ml of solution containing contaminant (perchlorate or hexavalent chromium) or 10 ml of HPLC grade water (controls) were added to each jar to create test concentrations of perchlorate at 3480, 6770, and 13,500 mg/L, and chromium at 21, 42, and 83 mg/L. Again, these concentrations were chosen to encompass the LC_{50} dose (Sorensen unpublished). After 24 h, surviving larvae were counted. Data were log transformed to fulfill the assumption of normality and log-dose probit curves were determined using probit analysis (Minitab, 2000).

1.3. Toxicity of Cr (VI) and perchlorate mixtures

The chronic toxicity of a mixture of perchlorate and Cr (VI) was tested by creating a solution containing the chronic LC_{50} of perchlorate (74 mg/L) plus the chronic LC_{50} of Cr (VI) (0.41 mg/L). Chronic mortality from these treatments was compared to mortality in LC_{50} controls for perchlorate and Cr (VI). An approximated LC_{50} level control was used as this provides the most reasonable flexibility; mortality can fluctuate without the risk of zero or 100% mortality (values that do not allow statistical analysis) (Zar, 1996). In addition, in log-dose probit models confidence intervals are the narrowest around the LC_{50} value (Finney, 1971). The expected percentage mortality (between 0 and 1), given the null hypothesis of an additive effect, was calculated using the formula $[E = O_a + O_b(1 - O_a)]$ where E is the expected mortality from the mixture, O_a is the observed mortality from compound a alone, and O_b is observed mortality from compound b alone (Finney, 1971; Salama et al., 1984). Values in this formula must be expressed as proportions (between 0 and 1), but can easily be converted back to percentages for easy interpretation. Using the calculated expected percentage mortality, a χ^2 test for homogeneity was performed to determine if the data could be pooled. A χ^2 test was then used to compare the observed mortality from the mixture to our calculated E value.

We used the following definitions, after Finney (1971) and Salama et al. (1984). If the combination of chemicals was statistically more toxic than expected from an additive process, the compounds were described as synergistic. If the combination was less toxic than expected, the compounds were considered antagonistic.

2. Results

2.1. Chronic toxicity

Slopes of log-dose probit lines for mortality from the two experimental dates were not significantly different for either contaminant as indicated by a χ^2 test for equal slopes (Minitab, 2000, perchlorate: $\chi^2 = 0.5573$, 1 df, $P = 0.455$; chromium: $\chi^2 = 0.8830$, 1 df, $P = 0.347$) and a t-test for equal elevation (Zar, 1996, perchlorate: $t = 0.0438$, 5 df, $P < 0.05$; chromium $t = 0.0852$, 5 df, $P < 0.05$), so the data were pooled, log transformed to fulfill the assumption of normality, and subjected to probit analysis. The LC_{50} for perchlorate was 74 ± 8.0 mg/L (mean $\pm$ SE), which was substantially higher than the LC_{50} for Cr (VI), 0.41 ± 0.15 mg/L (Table 1).

Growth was also significantly affected by the presence of perchlorate. As early as day 4 there were significant concentration-dependent differences in the RGI for contaminant concentrations as low as 21 mg/L vs. controls (ANOVA, $F_{4,15} = 13.52$, $P < 0.05$). The growth indices for the two highest concentration perchlorate treatments declined sharply at day 4, indicating an increase in mortality (Fig. 1). The subsequent increase in RGI at day 5 was a result of the surviving larvae successfully molting to the next instar. The RGI changed most appreciably during the first 5 days, after which RGI values stabilized for the remainder of the experiment.

Similar concentration-dependent reductions in growth were observed for Cr (VI) (Fig. 2). Starting at day 5, concentrations of 0.16 mg/L and higher significantly reduced the RGI (ANOVA, $F_{4,15} = 8.39$, $P < 0.05$). Like the perchlorate response, the greatest change in RGI occurred during the first 5 days of the test.

Wing length of mosquitoes was not significantly altered by levels of perchlorate up to 148 mg/L or by levels of Cr (VI) up to 0.47 mg/L. This was the case even when the data were corrected for sex and day of emergence. Averaged over all treatments, female wing length was 3.0 ± 0.18 mm (mean $\pm$ SD), while male wing length was 2.6 ± 0.17 mm. Thus, those individuals surviving to the adult stage did not appear to incur fitness costs, at least as measured by wing length, from development in contaminated water.

2.2. Acute toxicity

The acute LC_{50} level for perchlorate was $17{,}000 \pm 3200$ mg/L, approximately 230 times the LC_{50} recorded in the chronic tests (Table 1). Similarly, the acute LC_{50} for Cr (VI) was 38 ± 1.3 mg/L, nearly 100 times the chronic LC_{50} value (Table 1).

2.3. Joint toxicity

The predicted LC_{50} values for perchlorate (76 mg/L) and Cr (VI) (0.42 mg/L) resulted in 18% and 29% larval mortality, respectively, at day 10. Expected mortality from the mixture of perchlorate (74 mg/L) and Cr (VI) (0.41 mg/L) was calculated to be 41.8%. A χ^2 test for homogeneity indicated that the results could be pooled ($\chi^2 = 2.19$, 3 df, $P < 0.05$). After pooling the data, the observed mortality of the mixture ($56.2 \pm 9.46\%$, mean $\pm$ SE) was significantly greater than the

Table 1
Acute (24-h) and chronic (10-day) toxicity of perchlorate and Cr (VI) to *Culex quinquefasciatus* larvae

Compound	Length of test	Number of insects tested	LC_{50} (mg/L)	95% Confidence limits
Perchlorate	Acute	560	17,000	13,000–31,000
	Chronic	240	74	60–93
Hexavalent chromium	Acute	560	38	36–41
	Chronic	180	0.41	0.39–0.45

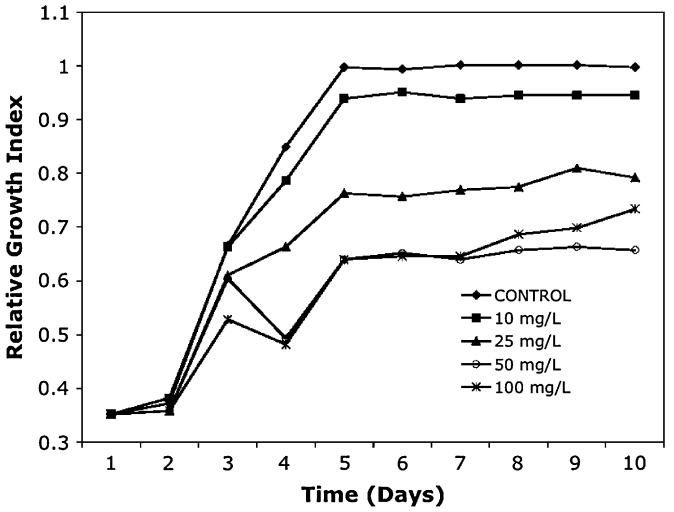

Fig. 1. Average Relative Growth Index of *Culex quinquefasciatus* subjected to various concentrations of perchlorate. Error bars indicate ±1 SE.

expected 41.8% ($\chi^2 = 6.29$, 1 df, $P < 0.05$), indicating that perchlorate and Cr (VI) interact synergistically, becoming more toxic than predicted by the action of either compound alone.

3. Discussion

3.1. Chronic toxicity

Widespread contamination of water with low concentrations (0.001 mg/L) of perchlorate is unlikely to affect *C. quinquefasciatus* survival. The perchlorate LC_{50} level (74 ± 8.0 mg/L) is 10,000 times higher than the perchlorate concentration in the Colorado River (approximately 0.006 mg/L, Hogue, 2003). However, there are some circumstances in which the properties of perchlorate, extreme solubility and stability and lack of volatility, may cause the contaminant to become extremely concentrated. These circumstances may warrant further investigation. First, evaporation of water from contaminated containers or puddles will result in evapoconcentration of perchlorate. Crop or backyard irrigation with daily or weekly watering in hot, dry desert climates such as found in the southwestern United States provides ideal circumstances for evapoconcentration. Secondly, given that plants can accumulate substantial amounts of perchlorate (e.g., lettuce accumulated 0.121 mg/kg, Sharp and Lunder, 2003; tamarisk 6 mg/kg, Urbansky et al., 2000), the transfer of perchlorate to water during leaf fall or plant death could be considerable.

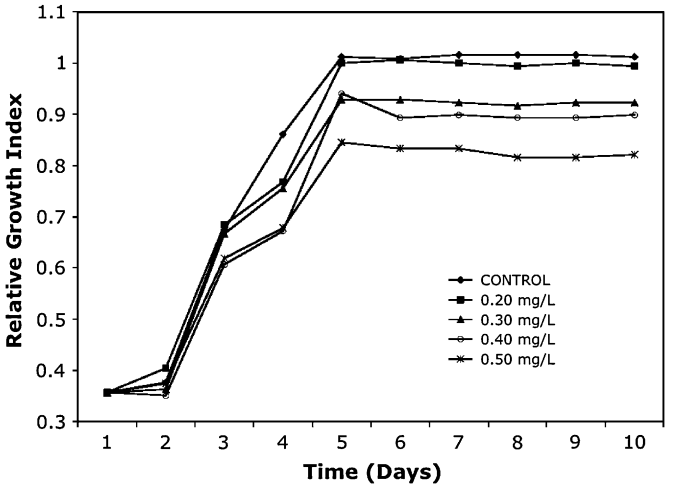

Fig. 2. Average Relative Growth Index of *Culex quinquefasciatus* subjected to various concentrations of chromium (VI). Error bars indicate ±1 SE.

The hexavalent chromium LC_{50} level (0.41 ± 0.15 mg/L) for *C. quinquefasciatus* was much lower than the LC_{50} level described by Trumble and Jensen (2004) for a terrestrial insect, *Megaselia scalaris* (Diptera: Phoridae) fed Cr (VI) in an artificial diet. The LC_{50} range for *C. quinquefasciatus* described in this study was within the range of concentrations encountered in contaminated surface water. The U.S. federal drinking water limit for total chromium is 0.1 mg/L, of which a varying proportion may be in the hexavalent form. We conclude that Cr (VI) contamination likely will affect mosquito populations, especially in situations where chromium (VI) undergoes evapoconcentration or where water is enriched with chromium through the addition of plant matter. Concentrations of Cr (VI) in some plants have exceeded 3170 mg/kg dry weight (Vajpayee et al., 1999).

Mosquito sensitivity to Cr (VI) is roughly comparable to the sensitivity of some freshwater fish such as *Oncorhynchus mykiss* (96 h LC_{50} of 0.11 mg/L, Castillo et al., 2000), *Pimephales promelas,* and *Ictalarus punctatus* (30 day LC_{50} values of 0.9 and 1.5 mg/L aqueous Cr (VI), respectively, Gendusa et al., 1993). Mortality of mosquitoes, and possibly other aquatic insects, due to Cr (VI) will impact fish and other predators by removing some fraction of available food resources. Additionally, metal contained in prey tissues may impact predators through biotransfer. Mosquito mortality is not likely to have broad implications for mosquito control, but may be an important consideration in specific local breeding sites with high levels of Cr (VI) contamination.

Development of *C. quinquefasciatus*, as measured by the RGI, was significantly slower when larvae were exposed to perchlorate or Cr (VI) pollution at levels as low as 25 mg/L or 0.20 mg/L, respectively. This finding has implications for larvae in temporary environments (irrigation pools, small containers in yards, etc.) because these habitats can disappear rapidly through evaporation, thereby killing any larvae that have not yet completed development. In addition, slower development means that larvae are exposed to other sources of mortality, such as predators and pathogens, for a longer time period.

For all perchlorate and Cr (VI) treatments, the RGI changed rapidly during the first 5 days, after which RGI values stabilized. This finding suggests that smaller larvae were affected more than larger larvae by both contaminants. Although the stronger initial response could also be the result of a rapid elimination of the susceptible fraction of the population, previous research has shown that instar is often a very important factor in determining mortality of invertebrates (Stuijfzand et al., 2000).

No significant difference in wing length of adults was observed for any of the perchlorate or chromium treatments

as compared to the control insects. Because wing length has been shown to correlate with body size and has been used as a fitness correlate (Armbruster and Hutchinson, 2002), these data suggest that surviving adults will not suffer a fitness loss as a result of perchlorate or chromium (VI) contamination. However, additional research on fecundity, longevity, and host-finding ability must be conducted before a definitive statement can be made regarding fitness.

3.2. Acute toxicity

The acute LC_{50} levels for perchlorate and Cr (VI) were 230 and 100 times the chronic LC_{50} levels, respectively. Therefore, bioassays using only acute lethality as an endpoint generate markedly different results than bioassays employing chronic or sublethal measures. Our results underscore the importance of carefully selecting toxicity endpoints appropriate to the questions being asked. Acute toxicity tests, when used to evaluate environmental risk, may suggest that a given concentration will have little or no effect when in fact the concentration is severely detrimental on a larger time scale. For example, the acute LC_{50} concentrations of Cr (VI) are unlikely to occur often in the environment, but the LC_{50} concentrations documented in the chronic studies frequently occur. Thus, even though acute toxicity is the most common measure reported for contaminant activity, especially against invertebrates (Stark and Banks, 2003), acute tests may not be best for establishing acceptable concentrations of pollutants.

3.3. Joint toxicity

Control treatments in this experiment containing approximated LC_{50} values of each pollutant resulted in 18% and 29% mortality, illustrating the variability in response across generations. Nonetheless these data were acceptable for use in characterizing the interaction of pollutants. Perchlorate and Cr (VI) interacted synergistically, with the combination of the two pollutants causing mortality approximately 14% greater than predicted by the action of each pollutant individually. However, in any water body with perchlorate and chromium (VI) present, these contaminants are likely to be joined by any number of other anthropogenic pollutants. When attempting to assess the toxicity of compounds at a specific site, it is important to consider not only the toxicity of individual chemicals, but also the interaction effects of mixtures. The mixture of perchlorate and Cr (VI) is but one of many possible combinations. If interaction effects are not taken into account, the environmental impacts of pollution may be severely underestimated. In order to gain a more comprehensive view of the specific impacts of perchlorate and chromium (VI) pollution on insect populations and ecosystems, future studies need to employ terrestrial insect consumers, such as herbivore or detritivore species that feed on plant material with high contaminant concentrations.

In toxicity testing, researchers need effective bioassay methods that can accurately determine toxicity economically and quickly. In our tests with *C. quinquefasciatus*, treatments that caused a significant change in RGI within the first 5 days also caused a significant decrease in survival by day 10. Shorter time-span studies lasting 10% or less of the lifespan of the organism, but longer than an acute study, are often referred to as subchronic toxicity studies. Subchronic studies with *C. quinquefasciatus*, using RGI as an endpoint, represent a promising method for a relatively inexpensive, rapid form of toxicity testing which will nonetheless yield more sensitive data than acute toxicity tests. Using this approach, some compounds could rapidly be eliminated for lack of biological activity, while pollutants that caused reductions in the RGI would be candidates for subsequent, more extensive chronic studies. This approach would serve to effectively target limited research funds to those pollutants with the greatest potential for population level effects.

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References

Armbruster, P., Hutchinson, R.A., 2002. Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatis*. Journal of Medical Entomology 39, 699–704.

Bays, J., 2002. Characterization of NH3 in anaerobic dairy lagoons. <http://www.cmer.wsu.edu/Summer/bays.pdf>.

Benvenga, S., Alesci, S., Trimarchi, F., Facchiano, A., 1999. Homologies of the thyroid sodium-iodide symporter with bacterial and viral proteins. Journal of Endrocrinological Investigation 22, 535–540.

Canivet, V., Chambon, P., Gilbert, J., 2001. Toxicity and bioaccumulation of arsenic and chromium in epigean and hypogean freshwater macroinvertebrates. Archives of Environmental Contamination and Toxicology 40, 345–354.

Castillo, G.C., Vila, I.C., Neild, E., 2000. Ecotoxicity assessment of metals and wastewater using multitrophic assays. Environmental Toxicology 15, 370–375.

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

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

Finney, D.J., 1971. Probit Analysis, third ed. Cambridge University, London.

Forbes, V.E., Calow, P., 1999. Is the per capita rate of increase a good measure of population-level effects in ecotoxicology? Environmental Toxicology and Chemistry 18, 1544–1556.

Gendusa, T.C., Beitinger, T.L., Rodgers, J.H., 1993. Toxicity of hexavalent chromium from aqueous and sediment sources to *Pimephales promelas* and *Ictalurus punctatus*. Bulletin of Environmental Contamination and Toxicology 50, 144–151.

Goleman, W.L., Carr, J.A., Anderson, T.A., 2002a. Environmentally relevant concentrations of ammonium perchlorate inhibit thyroid function and alter sex ratio in developing *Xenopus laevis*. Environmental Toxicology and Chemistry 21, 590–597.

Goleman, W.L., Urquidi, L.J., Anderson, T.A., 2002b. Environmentally relevant concentrations of ammonium perchlorate inhibit development and metamorphosis in *Xenopus laevis*. Environmental Toxicology and Chemistry 21, 424–430.

Hogue, C., 2003. Rocket-fueled river. Chemical and Engineering News August 18, 2003, 37.

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

Leslie, H.A., Pavluk, T.I., Vaate, A., Kraak, M.H.S., 1999. Triad assessment of the impact of chromium contamination on benthic macroinvertebrates in the Chusovaya river. Archives of Environmental Contamination and Toxicology 37, 182–189.

Lifsher, Marc, 2004. PG&E's toxic plume creeps toward L.A. water supply. Los Angeles Times, 6 March 2004.

Los Angeles Regional Water Quality Control Board, 2004. <http://www.swrcb.ca.gov/rwqcb4/html/water_qty/chromium_S1.html>.

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

Mulla, M.S., Darwazeh, H.A., 1988. Efficacy of new insect growth regulators against mosquito larvae in dairy wastewater lagoons. Journal of the American Mosquito Control Association 4, 322–325.

Salama, H.S., Foda, M.S., Zaki, F.N., Moawad, S., 1984. Potency of combinations of *Bacillus thuringiensis* and chemical insecticides on *Spodoptera littoralis* (Lepidoptera: Noctuidae). Journal of Economic Entomology 77, 885–890.

Savage, H., Miller, B., 1995. House mosquitoes of the U.S.A., *Culex pipiens* complex. Wing Beats 6, 8–9.

Sharp, R., Lunder, S., 2003. Enviornmental Working Group Report: suspect Salads. <http://www.ewg.org/reports/suspectsalads/es.php>.

Smith, P.N., Theodorakis, C.W., Anderson, T.A., Kendall, R.J., 2001. Preliminary assessment of perchlorate in ecological receptors at the Longhorn Army Ammunition Plant (LHAAP), Karnack, Texas. Ecotoxicology 10, 305–313.

Smith, S., Peterson, P.J., Kwan, K.H.M., 1989. Chromium accumulation, transport and toxicity in plants. Toxicology and Environmental Chemistry 24, 241–251.

Sorensen, M.A., Trumble, J.T., 2004. Bioavailability and bioremediation of perchlorate salts. In: Bodari (Ed.), Recent Research Developments in Environmental Biology, pp. 349–363. Trivandrum, India.

Stark, J.D., Banks, J.E., 2003. Population-level effects of pesticides and other toxicants on arthropods. Annual Review of Entomology 48, 505–519.

Statview, 2001. SAS Institute, Cary, North Carolina. Version 5.0.1.

Stuijfzand, S.C., Poort, L., Greve, G.D., van der Geest, H.G., Kraak, M.H.S., 2000. Variables determining the impact of diazinon on aquatic insects: taxon, developmental stage, and exposure time. Environmental Toxicology and Chemistry 19, 582–587.

Trumble, J.T., Jensen, P.D., 2004. Ovipositional response, developmental effects and toxicity of Cr (VI) to *Megaselia scalaris*, a terrestrial detritivore. Archives of Environmental Contamination and Toxicology 46, 372–376.

US EPA, 2005. <http://www.epa.gov/fedfac/documents/perchlorate_map/nationalmap.htm>.

US EPA, 1998. Drinking Water Contaminant Candidate List. Washington, DC, Doc No EPA/600/F-98/002.

US EPA, 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. NCEA-1–0503.

Urbansky, E.T., Magnuson, M.L., Kelty, C.A., Brown, S.K., 2000. Perchlorate uptake by salt cedar (*Tamarix ramosissima*) in the Las Vegas Wash riparian ecosystem. Science of the Total Environment 256, 227–232.

Vajpayee, P., Sharma, S.C., Tripathi, R.D., Rai, U.N., 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.

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

Wolff, J., 1998. Perchlorate and the thyroid gland. Pharmacological Review 50, 89–105.

World Bank, 2002. <http://www.worldbank.org/nipr/polmod.htm>.

Yang, R.S.H., 1994. Toxicology of chemical mixtures. Case Studies, Mechanisms and Novel Approaches. Academic Press, New York.

Zar, J.H., 1996. Biostatistical Analysis, third ed. Prentice-Hall. Inc., New Jersey, USA.

Zhang, J., Li, X., 1987. Chromium pollution of soil and water in Jinzhou. Journal of Chinese Preventative Medicine 21, 262–264.

Zhang, M., Chaudhuri, S.K., Kubo, I., 1993. Quantification of insect growth and its use in screening of naturally occurring insect control agents. Journal of Chemical Ecology 19, 1109–1118.