Lethal and Sublethal Effects of Chlorine, Phenol, and Chlorine-Phenol Mixtures on the Mud Crab, *Panopeus herbstii*

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The mud crab, *Panopeus herbstii*, was acutely exposed (96-hr) to chlorine-produced oxidants (CPO), phenol, and a CPO–phenolic mixture (1:1) to determine lethal and sublethal effects. The 96-hr (LC₅₀) values were determined for each individual compound and mixture. Additionally, whole-animal respiration rates were measured following acute exposure to sublethal concentrations of each compound or mixture. Phenol uptake/decomposition rates were measured in the phenol and CPO–phenol mixture concentrations.

Results indicated 96-hr LC₅₀ values of 1.06 mg/L for CPO (fiducial limits (FL) = 0.53 – 2.01 mg/L), 52.8 mg/L for phenol (FL = 45.6 – 64.5 mg/L), and 184.7 mg/L total toxicant units (TTU) for the CPO–phenol mixture (FL = 143.7 – 250.2 mg/L TTU). Statistical analysis indicated that the acute toxicity of the CPO–phenol mixture was less than additive.

Sublethal studies indicated that only acute exposure to sublethal concentrations of CPO caused altered respiration rates. After 96-hr decomposition, metabolic rates in all CPO-exposure crabs generally returned to control rates. Uptake and depuration rate studies indicated significantly lower phenol uptake rates in crabs exposed to the CPO–phenol mixture. These findings suggest that the less-than-additive toxicity of the CPO–phenol mixture may result from lowered uptake/decomposition rate kinetics and indicate that the discharge of chlorinated-phenolic waste may not result in additive and/or synergistic interactions, but rather in less-than-additive effects on decapod aquatic species.

**Introduction**

The chlorination of drinking water and sewage effluent may result in the formation of many chemical compounds, including chlorine-produced oxidants (CPOs), such as hypochlorous acid (HOCI), hypochlorite (OCl⁻) in freshwater; hypobromous acid (HOBr), hypobromite (OBr⁻) in saltwater; trihalomethanes (THMs), such as CHCl₃ in freshwater, bromoform (CHBr₃) in saltwater; haloacetonitriles and chlorinated amines (1–4). Other chemical compounds in water and sewage that serve as precursor molecules for the *de novo* synthesis of chlorinated compounds have clearly been documented (5–7). The presence of these compounds in drinking water may cause cancer in man and has been studied extensively (8–10). Additional concern has been focused on the discharge of these compounds into aquatic habitats.

CPO and phenol are two compounds associated with industry, human waste disposal, and general population use. In Maryland, an estimated $13.3 \times 10^6$ kg chlorine is discharged into the Chesapeake Bay each year from sewage treatment plants and power plants (11). Phenol concentrations in industrial effluents of less than 1 to 3,016,000 µg/L have been reported, and levels ranging from 70 to 100 µg/L for raw sewage and 6 to 12 µg/L for sewage effluent have been reported (12).

The chlorination of sewage effluent may result in the formation of chlorinated phenolic compounds such as 2-chlorophenol, 4-chlorophenol, 2,6-dichlorophenol, and 2,4,6-trichlorophenol in freshwater (1,13). If chlorine–phenol interactions occur in seawater, brominated phenolics, consisting predominantly of 2,4,6-tribromophenol and lesser amounts of 2,4- and 2,6-dibromophenol, are formed (14). The discharge of halogenated phenolic compounds into aquatic ecosystems may result in potentially adverse effects on sensitive species.

Although numerous studies have documented the adverse individual effects of CPO and phenols on aquatic organisms (15–20), few very studies have examined and compared the adverse effects of halogenated phenolic mixtures. The purpose of this study was to compare the lethal (LC₅₀) and sublethal (respiration and phenol uptake) effects of acute exposure to CPO, phenol, and a

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CPO–phenol mixture on the estuarine mud crab, Panopeus herbstii.

Material and Methods

Collection and Acclimation

P. herbstii were collected at Bohicket Creek (coordinates N 32° 36′; W 80° 15′), which is a tidal tributary of the North Edisto River estuary in South Carolina. Collected crabs were transported back to the laboratory and acclimated for a minimum of 14 days to 25°C, 25 ppt salinity and a 12-hr light-dark cycle. During acclimation, crabs were fed either minced oyster or Tetramin Fish Flakes with water changes made twice weekly.

Toxicity Tests

The 96-hr toxicity tests were conducted at 25°C and 25 ppt salinity using adult crabs exposed to CPO (0.05 to 4.00 mg/L), phenol (1.00 to 160.00 mg/L), and 1:1 CPO–phenol mixture (2.00 to 120.00 mg/L).

All toxicity tests were static with water changes made daily. An additional unexposed group was maintained as a control. For each exposure concentration and control group, three replicates were used (N = 12–18).

The chloride as sodium hypochlorite (NaOCl) and phenol (C₆H₅OH) used in preparation of the CPO, phenol, and CPO–phenol mixture stocks were reagent- and technical-grade chemicals, respectively (Fisher Scientific Company). CPO concentrations were measured using a Fisher CL Titrimeter (Model 397). Reagents used for titrations were pH 4 buffer, potassium iodide (KI), and phenylarsine oxide (Fisher Scientific Company). CPO concentrations (mg/L) were measured using methods outlined in the literature (21). Phenol concentrations reported are nominal concentrations (mg/L). The CPO–phenol mixture concentrations are nominal concentrations reported as total toxicant units (TTU) in mg/L (22).

Sublethal Studies

Oxygen consumption (μL O₂/g/hr) was measured in crabs before and after acute (96-hr) exposure to CPO (0.13, 1.65, and 4.00 mg/L), phenol (1, 16, and 32 mg/ L), and the CPO–phenol mixture (2, 20, and 80 mg/L T TU). Following exposure, crabs were depurated in uncontaminated seawater (25°C and 25 ppt salinity) for 96 hr, and respiration measurements were repeated. Respiration was measured using a Gilson Differential Respirometer.

The uptake of radiolabeled [14C]phenol (μg/g) was measured following acute (96-hr) exposure of mud crabs to phenol (2 mg/L) and the chlorine–phenol mixture (2 mg/L phenol or 4 mg/L T TU). Measurements were taken at 24, 48, and 96 hr of exposure and depuration for the CPO–phenol mixture. For phenol, measurements were taken at 24 and 96 hr of exposure and 96 hr of depuration. Unexposed control groups were also measured in both tests. The radiolabeled solution was made by adding 100 μL of 0.5 μCi [14C]phenol to the 2 mg/L phenol and CPO–phenol mixture. A minimum of four crabs was randomly selected at each sampling time, and gills, hepatopancreas, and muscle tissues were dissected and analyzed individually. A 1-mL portion of each diluted (10:1), homogenized tissue was placed in a 20-mL vial with 0.5 mL of Soluene-350 solubilizer. The vials were heated at 60°C for 24 hr, after which 10 mL of Aquasol-2 was added. Each vial was counted for 10 min using a Packard Tri-Carb 460 C Liquid Scintillation System (23).

Statistics

The statistical analyses consisted of analysis of variance and Scheffé’s multiple comparisons for respiration measurements and radiolabeled phenol uptake measurements (24). The probit procedure was used with the toxicity data (25,26). The mean, standard deviation, and standard error were determined for all three data sets. A p value less than 0.05 was the minimum level of statistical significance accepted.

Results

Toxicity Tests

Results of toxicity tests are depicted in Figure 1. These results indicated 96-hr LC₅₀ values of 1.07 mg/L (fiducial limits (FL) = 0.53 to 2.01 mg/L) for CPO, 52.8 mg/L (FL = 45.6 to 64.8 mg/L) for phenol, and 184.7 mg/L T TU for the CPO–phenol mixture (FL = 143.7 to 250.2 mg/L T TU). CPO was considerably more toxic than phenol or the CPO–phenol mixture, and phenol was more toxic than the CPO–phenol mixture. Statistical analysis indicated that the joint toxicity of the CPO–phenol mixture was less than additive when compared to the individual toxicity of CPO and phenol.

Oxygen Consumption

CPO exposure significantly reduced respiration rates, compared to those of controls, in crabs at all exposure concentrations tested (Fig. 2), whereas phenol exposure had no significant effect on respiration at all concentrations tested (Fig. 3). The CPO–phenol mixture significantly reduced respiration rates in exposed crabs when compared to those of controls but only at the highest concentration (80 mg/L T TU) tested (Fig. 4). Additional statistical analysis indicated that respiration rates in crabs exposed to all CPO concentrations and to the highest CPO-phenol concentration were significantly lower than respiration rates in crabs exposed to all phenol concentrations and to the lower concentrations of the CPO–phenol mixture. These results suggest that CPO may adversely affect whole-animal metabolic rates at low concentrations (0.13 mg/L). When CPO was combined with phenol, effects on respiration were noted only when nominal CPO and phenol concentrations were 40 mg/L (80 mg/L T TU).
Following 96-hr depuration, respiration rates in all exposure crabs had returned to control values except in crabs exposed to the lowest CPO concentration (0.13 mg/L), in which respiration remained depressed (Fig. 2-4). These results suggest that the effect of CPO, phenol, and the CPO–phenol mixture exposure on respiration was generally labile.

Uptake of Phenol

The highest rate of phenol uptake was observed in gill tissue, followed by hepatopancreas and muscle for both the phenol (Fig. 5) and the CPO–phenol exposure (Fig. 6). Maximum total tissue concentrations of phenol were 17.50 µg/g for phenol and 7.91 µg/g for the CPO–phenol mixture. Maximum bioconcentration factors were 8.75 for phenol and 3.95 for the CPO–phenol mixture. Statistical analysis indicated that uptake was only significantly (p < 0.05) higher in gill tissue from phenol-exposed crabs when compared to that in gill tissue from crabs exposed to the CPO–phenol mixture. These results suggest that exposure to the CPO–phenol mixture may inhibit the uptake of phenol, possibly because of interference with the permeability of the gill.

Following 96-hr depuration, phenol was rapidly but incompletely lost. Highest depuration rates were observed in the gills and hepatopancreas. Maximum total tissue concentrations following 96-hr depuration were 9.09 µg/g for phenol. This concentration represented a 48% depuration in 96 hr.

Following 96-hr exposure, body burdens did not significantly decline, but rather slightly increased in the crabs exposed to the CPO–phenol mixture. This increase was attributed to a possible metabolic effect of chlorine on gill membrane permeability. Although this effect may have obscured any obvious comparisons between depuration and exposure periods, the results do suggest slower depuration rate kinetics for the CPO–phenol exposure group when compared to that of phenol-exposed crabs.
Discussion

The discharge of chlorine and phenols into aquatic environments may result in the exposure of many invertebrates and fish. Very few studies have examined the potential interactions of CPO and phenol on aquatic organisms, even though these two compounds are often discharged together into aquatic environments (12–14).

In this present study, CPO was found to be more toxic to the mud crab, *P. herbstii*, than phenol. Similarly, phenol was found to be more toxic than the CPO–phenol mixture. The CPO–phenol mixture had less-than-additive acute toxicity. This finding suggests that the interaction of phenol and chlorine in the marine environment produces a complex mixture that is less toxic to mud crabs than its individual CPO (HOBr, OBr−) or phenol components. Chemical studies suggest that the interaction of CPO and phenol in seawater would produce a mixture of predominantly (40–50%) 2,4,6-tribromophenol (27), with lesser amounts of 2,4- and 2,6-dibromophenols (14). The present study suggests that these brominated phenolic forms, probably present in the CPO–phenol mixture, were less toxic than phenol alone in seawater. Indications are that halogenation of the phenolic ring may result in less acutely toxic compounds. These results contrast sharply with those from earlier studies (16) on freshwater species, which reported increased acute toxicity of chlorinated phenols in fathead minnows. In this freshwater study 96-hr LC50 for pure phenol was 28 mg/L compared to 8.6 mg/L for 2,4,6-trichlorophenol. However, our study clearly indicates a significant reduction in toxicity to mud crabs among the CPO–phenolic mixtures.

Results from sublethal studies of respiration rate indicated that CPO exposure significantly reduced oxygen consumption. Earlier studies (28–32) have shown reduced oxygen consumption among estuarine invertebrates at concentrations ranging from 10 to 10,000 μg/L. Phenol exposure did not have a significant effect on respiration. The CPO–phenol mixture affected respiration only at the highest concentration tested (80 mg/L TTU).

After depuration, respiration rates in all treatments, except the 0.13 mg/L CPO concentration, were similar to control values, suggesting that the effects of CPO,
phenol, and CPO-phenol mixtures are generally acute and very labile. These findings differ from the results of earlier studies by Scott et al. (33,34), who reported significant chronic effects on respiration in oysters 15 days after exposure to CPO.

Results from radiolabeled [14C]phenol uptake studies indicated reduced uptake/depuration rate kinetics in the CPO-phenol mixture when compared to the rate kinetics of phenol alone. Exposing crabs to the CPO-phenol mixture did not alter or reduce their metabolism; rather, metabolic activity was normal. This lack of correlation with physiological effects suggests that uptake/depuration differences between the CPO-phenol mixture and phenol alone may be directly related to chemical differences between halogenated phenols in the mixture and phenol alone. These findings also suggest that halogenated phenols would be bioconcentrated and depurated at lower rates than phenol. These results differ from those of earlier studies (19,23), which reported enhanced phenol uptake in mud crabs exposed to coal-oil, water-soluble fractions, and enhanced phenol uptake in fiddler crabs in the presence of Kepone. The results of these earlier studies indicated that exposure to a phenol mixture may cause changes in the permeation rate of the cell membrane, thus affecting uptake/depuration rates. The lower uptake/depuration rates in the CPO-phenol mixture may have resulted from similar membrane effects. Results from other studies on CPO effects (35,36) have indicated alterations in osmoregulation in exposed crabs. Similar osmoregulation effects may have also occurred in the CPO-phenol mixture, thus altering uptake/depuration rate kinetics.

Future efforts should be directed at assessing the acute and chronic toxicity of chlorinated phenolic mixtures. During this present study, severe, gross histopathological changes were observed in the hepatopancreas of crabs exposed to phenol and the CPO-phenol mixture. Chronic toxicity studies may better evaluate the overall toxicological potential of chlorinated phenolics on aquatic organisms. Additional research will also be required to determine if the differences in uptake/depuration kinetics are related to chemical speciation differences in halogenated phenolics or due to altered membrane permeability and osmoregulation effects.
We gratefully acknowledge the financial support of the Department of Environmental Health Sciences, University of South Carolina.

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