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Toxicity of Ozonated Estuarine Water to Juvenile Blue Crabs (*Callinectes sapidus*) and Juvenile Atlantic Menhaden (*Brevoortia tyrannus*)

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Large quantities of estuarine and marine water are treated with chlorine to prevent condenser system fouling at power plants, while a variety of municipal and waste water treatment processes discharge chlorinated effluents into marine and estuarine environments. Chlorine and its residual by-products, however, are toxic to many forms of aquatic life (BRUNGS 1976; HALL et al., in press a & b). Consequently, this has led to the consideration of alternative oxidants (U. S. ENVIRONMENTAL PROTECTION AGENCY 1976; BURTON & LIDEN 1978; GAREY 1980). Ozone is one alternative oxidant which has proven to be an effective biocide and disinfectant in many fresh water applications (RICE & BROWNING, 1973). Ozonation of estuarine and marine waters, however, may produce residual compounds similar to those produced by chlorination (EPPLEY et al. 1976; HELZ in press).

This study was initiated to provide baseline information on the toxicity of ozonated estuarine water to two representative estuarine species. The blue crab, *Callinectes sapidus* Rathbun, and the Atlantic menhaden, *Brevoortia tyrannus* Latrobe, were selected because of their wide distribution and commercial importance. The toxicity of ozone has been compared with chlorine toxicity data from the literature in an effort to examine possible similarities in toxicity. In this paper the nomenclature ozone-produced oxidant (OPO) and chlorine-produced oxidant (CPO) is used to refer to the total residual oxidant concentration, measured amperometrically as "total residual chlorine", produced in solution following ozonation or chlorination, respectively. The term CPO, therefore, is synonymous with the more common terms total residual chlorine (TRC) and total residual oxidant (TRO).

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MATERIALS AND METHODS

Blue crabs and Atlantic menhaden were held in the laboratory in large 800-liter fiberglass tanks supplied with a continuous flow of 25°C filtered Patuxent River water for at least two weeks prior to experimentation. During acclimation, blue crabs were fed shrimp while Atlantic menhaden were fed finely ground Purina trout chow. Mortality during the acclimation period was minimal for Atlantic menhaden after the first two days; the majority of losses for blue crabs were mainly due to cannibalism.

Twenty-five crabs (27.3 ±8.7 mm; 2.3 ±2.1 g) were continuously exposed to each OPO concentration of 0.000, 0.113 (±0.031), 0.232 (±0.031), 0.303 (±0.037), 0.397 (±0.078), 0.529 (±0.040), 0.628 (±0.088) and 0.674 (± 0.208) mg/l (as total residual chlorine) for 144 hours in 56-liter all glass aquaria. Mortality observations were made after 6, 8, 12, 18, 24, 36, 48, 60, 72, 96, 120 and 144 hours exposure. Eighty Atlantic menhaden were continuouly exposed to each OPO concentration of 0.000, 0.079 (±0.012), 0.120 (±0.026), 0.209 (±0.018)and 0.324 (±0.018) mg/l (as total residual chlorine) for 96 hours in 110-liter all glass aquaria. Mortality was checked following 12, 24, 48, 72 and 96 hours of exposure. Crabs and Atlantic menhaden were not fed during the experiments.

Gaseous ozone was produced by passing high quality aviator oxygen through a Welsbach T-816 ozonator (Welsbach Ozone Systems Corp., Philadelphia, PA). The resulting ozone-oxygen mixture (≅ 4% ozone by weight) was bubbled into the base of an 11.5-liter, polycarbonate, counter current contact column (7 cm I.D.; 2.75 m high) at 1.5 liter/min through a 3 cm frittered glass disc. Ozonated water was drawn from the base of the contact column and metered into the water supply lines of the exposure chambers through calibrated glass rotameters. Thus, the desired exposure concentrations were produced by mixing non-ozonated water from a head tank with a much smaller volume of ozonated water from the contact column.

OPO concentrations were measured amperometrically as equivalent total residual chlorine (AMERICAN PUBLIC HEALTH ASSOCIATION et al. 1976) using a PAR polarographic analyzer (Model 174A; Princeton Applied Research Corp., Princeton, NJ). The titration cell had a 10-g rotating, spiral platinum active electrode and a standard platinum disc reference electrode. Phenylarsine oxide (2.82×10^{-3} N) was the titrant and was delivered to the titration cell with a syringe microburett (Model SB2; Micro-Metric Instrument Co., Cleve-

land, OH). Ph 4 buffer and potassium iodide reagents were premixed in the bottom of the sample collection vessel to minimize possible underestimation of the residual oxidant concentration (CARPENTER et al., 1977; CRECELIUS et al., 1978).

Mean water quality (±S.D.) for these studies was: temperature = 25.2 (±0.4)°C, salinity = 7.4 (±0.3) °/oo, and dissolved oxygen concentrations which ranged from 4.5 to 10.0 mg/l across the OPO test concentrations.

Statistical Analysis

Response surface models were derived for both juvenile blue crabs and juvenile Atlantic menhaden using an arcsine transformation and the stepwise regression procedure of SAS (SAS INSTITUTE INC. 1979). The following linear model was initially introduced to this program:

$$\text{Arcsine}\,[(r/n)^{0.5}] = a + b(C) + c(T) + d(D) + e(C^2) + f(T^2) + g(DC) + h(DT) + i(D^2) + j(\log C) + k(\log T)$$

where

C = exposure concentration (mg/l)
T = exposure time (hours)
D = dose = C x T
r = mortality response at a given dose
n = sample size at a given dose
a-k = coefficients estimated by the least squares procedure

Only those variables making a significant ($p < 0.05$) contribution to the final model were selected. In addition to the response surface models a SAS probit analysis (SAS INSTITUTE INC. 1979) was used to calculate LC50 values for which 95% confidence limits could be calculated and thus compared quantitatively with the literature.

RESULTS AND DISCUSSION

The predictive equations for the mortality of juvenile blue crabs and juvenile Atlantic menhaden exposed to OPO are shown in Table 1. These equations calculate transformed predicted mortality values (Y) which must be back-transformed to find the actual percent mortality using the following equation:

$$\text{Predicted percent mortality} = (\sin Y)^2 \times 100$$

These back-transformed predicted percent mortality values were used to construct the response surfaces in Figures 1 and 2.

TABLE 1

Predictive Response Models for Mortality of Juvenile Blue Crabs and Juvenile Atlantic Menhaden Exposed to a Series of OPO Concentrations at 25°C.

Species	Modeling Equation*
Blue Crab	$Y = -0.9551 + 2.4964\ C + 0.2272\ (\log T)$
Menhaden	$Y = 0.0063 - 0.0122\ D + 0.0976\ DC$

*C = OPO concentration in mg/l.
*T = Exposure time in hours.

The modeling equation for juvenile blue crabs (Table 1) indicates that OPO concentration (C) and the natural logarithm of time (log T) are the only significant ($p < 0.10$) independent variables for predicting the mortality of this species during continuous exposure to OPO. This is clearly shown in Figure 1 where mortality increases logrithmically parallel to the exposure time axis and in the flattened sigmoid fashion characteristic of the arcsine function along the concentration axis. Both terms made highly significant ($p < 0.0001$) contributions to the model. However, a comparison of partial F-values showed concentration to be the most important of the two variables. The modeling equation for juvenile Atlantic menhaden, however, shows that the mortality of this species is significantly affected by the dose ($p < 0.0163$) and dose x concentration ($p < 0.0001$) terms. Both partial F-values and significance levels proved the dose x concentration term to be the most important variable in predicting the toxicity of OPO to juvenile menhaden.

Table 2 lists the LC50 values for both blue crabs and Atlantic menhaden calculated from both the modeling equations and repeated probit regression. In all cases except one (blue crab, 6-h LC50), the LC50 computed from the modeling equation fell well within the 95% confidence limits derived from standard probit techniques. LC50 values for the Atlantic menhaden, however, should be viewed with caution since they have been extrapolated beyond the upper concentration limit of the experiment.

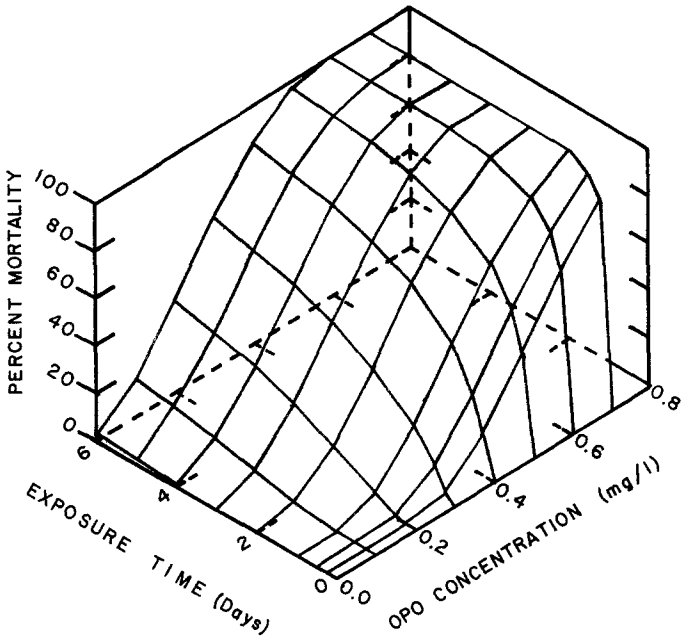

Figure 1. Mortality response surface for juvenile blue crabs exposed to a series of OPO concentrations for periods up to 144 hours.

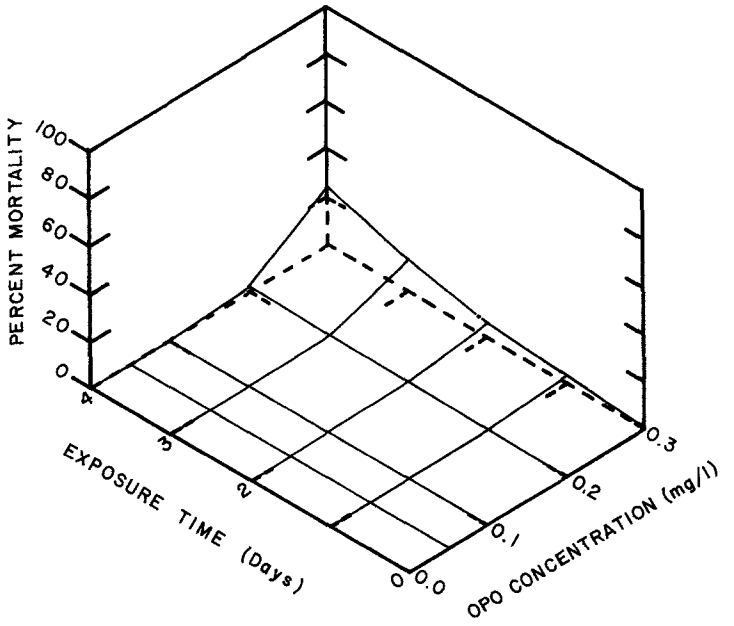

Figure 2. Mortality response surface for juvenile Atlantic menhaden exposed to a series of OPO concentrations for periods up to 96 hours.

TABLE 2

Predicted LC50 Values Calculated from Response Surface Models and Repeated Probit Analyses for the Toxicity of OPO to Juvenile Blue Crabs and Juvenile Atlantic Menhaden.

Species	Exposure Time (h)	Predicted LC50 Model	Predicted LC50 Probit	95% Confidence Limits†
Blue Crab	6	0.53	0.63	0.590-0.698
	12	0.47	0.48	0.452-0.513
	18	0.43	0.43	0.350-0.508
	24	0.41	0.39	0.339-0.450
	36	0.37	0.36	0.306-0.437
	48	0.35	0.35	0.276-0.449
	60	0.33	0.32	0.290-0.349
	72	0.31	0.30	0.269-0.321
	96	0.28	0.26	0.160-0.308
	120	0.26	*	* - *
	144	0.25	*	* - *
Menhaden	24	-	-	- - -
	48	0.48	0.42	0.370-0.590
	72	0.40	0.38	0.351-0.464
	96	0.36	0.36	0.335-0.400

* Insufficient data for analysis.
† For probit analysis only.

Most available literature on the toxicity of ozone has been centered around fresh water studies and/or with salt water bacterial, phytoplankton or zooplankton species. TONER & BROOKS (1975) studied the effects of very short exposures (1-10 min) of crab zoea and megalops (species not given) and Atlantic silversides (*Menidia menidia*) exposed to OPO concentrations ranging from 0.08-0.65 mg/l. The crab zoea and megalops and the Atlantic silversides in their study were much more sensitive than the species tested in our study. They found for both crabs and Atlantic silversides that exposure to OPO concentrations ≧ 0.2 mg/l for a time ≧5 min caused 80-100% mortality when measured 48 hours following exposure. These results are not entirely contradictory, however, since early developmental stages of many invertebrates and fish are generally more sensitive than later stages and Atlantic silversides are extremely sensitive fish relative to other fish when exposed to many toxicants. RICHARDSON et al. (with editor) have shown that the acute toxicity of OPO to the early developmental stages of the American

oyster (*Crassostrea virginica*) are also more sensitive (96-h LC50 = 0.12 mg/l OPO) than the crabs and Atlantic menhaden in this study while the adult oyster is much less sensitive.

The chemistry of oxidants in sea water is extremely complex and a number of authors have shown that a variety of physical and chemical variations in water quality may result in very different residual by-products whose exact speciation and concentrations are unknown. However, recent studies on the chemistry of ozonation and chlorination of estuarine and marine waters have indicated that these two oxidants may produce similar residual oxidant species (EPPLEY et al. 1976; HELZ in press). In addition it appears that the mode of toxic action for both OPO and CPO is through destruction of the gill epithelium and the oxygen carrying components of the blood (ZEITOUN 1977; BLOCK et al. 1978, WEDEMEYER et al. 1979; MIDDAUGH et al. 1980). Thus, the mechanisms of toxicity of OPO and CPO to estuarine crustacea and fish may be similar. This hypothesis is supported by available CPO toxicity data from the literature. ROBERTS (1976) reported 48- and 96-h LC50's of 0.42 and 0.32 mg/l CPO, respectively, for juvenile blue crabs. These values are not significantly different from our 48- and 96-h LC50's of 0.35 (0.276-0.449) and 0.26 (0.160-0.308) mg/l OPO, respectively, for the blue crab experiment. LIDEN et al. (1980) found 100% survival for juvenile Atlantic menhaden exposed to 0.062 mg/l CPO for 19 days which agrees well with our data (Figure 2).

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