An Evaluation of Acute Bromine Chloride Toxicity to Grass Shrimp (*Palaemonetes* spp.) and Juvenile Blue Crabs (*Callinectes sapidus*)*

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INTRODUCTION

Bromine chloride has recently been proposed as an alternative to chlorine for disinfection of municipal wastewaters and the prevention of biofouling in industrial cooling water systems (JACKSON, 1974; MILLS, 1976; WACKENHUTH and LEVINE, 1974 and MS in press). Alternatives to chlorine are being pursued because of the toxicity of low level concentrations of chlorine and its residual by-products to aquatic organisms (see reviews by BRUNGS, 1974 and 1976 and TSAI, 1975). A recent Environmental Task Force has recommended that the exclusive use of chlorine not be continued where protection of aquatic life is of primary concern (U. S. ENVIRONMENTAL PROTECTION AGENCY, 1976).

Although several studies have shown that bromine chloride can be used as a substitute for chlorine in waste treatment and cooling water processes, little information is available on the environmental acceptability of bromine chloride and its residual by-products discharged to aquatic environments. Only one comprehensive study has been conducted on two freshwater species (ANONYMOUS, 1974); no published data are available on the toxicity of bromine chloride and its residuals to estuarine and marine species. This study was initiated to provide baseline information on the toxicity of bromine chloride to two important estuarine macroinvertebrates; the grass shrimp and the blue crab.

MATERIALS AND METHODS

Post-larval grass ghrimp (*Palaemonetes* spp.; primarily *P. pugio*) were collected from the Patuxent River near Benedict, Maryland (HOLTHUIS, 1952). Juvenile blue crabs (*Callinectes sapidus*) were collected from Chesapeake Bay, Calvert County, Maryland. The mean wet weight (± S.D.) and length of the grass

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shrimp used in the actual experimental tests were 0.2 (± 0.11) g and 2.9 (± 0.41) cm, respectively. The mean wet weight of the blue crabs was 1.1 (± 0.43) g with a width of 2.6 (± 0.51) cm. Both species, which included males and females, were held in the laboratory for a minimum of two weeks in separate aquaria supplied with a continuous flow of well aerated Patuxent River water. Basic water quality during the two week acclimation period and experimental runs was as follows: mean temperature (± S.D.) 19.0 (± 1.09) ^{o}C; salinity 6.9 (± 0.45) $^{o}/oo$; dissolved oxygen 8.5 (± 0.59) mg/1; pH 7.8 (± 0.32) and ammonia-N 0.18 (± 0.083) mg/1. A cyclic photoperiod of 12L:12D was maintained during acclimation and experimental study. Grass shrimp were maintained on detritus and finely chopped fish (*Brevoortia tyrannus*) added daily. Blue crabs were fed *B. tyrannus* daily. No animals were fed 24 hours prior to or during an experiment.

The acute toxicity of bromine chloride to non-molting post-larval grass shrimp and non-molting juvenile blue crabs was determined by standard continuous flow bioassay procedures outlined in *Standard Methods* (AMER. PUBLIC HEALTH ASSOC., 1976). Groups of 20 shrimp and 10 crabs were exposed continuously for periods up to 96 hours to the following test concentrations: 0.15 mg/1 total residual bromine chloride (TRB), 0.30 mg/1 TRB, 0.50 mg/1 TRB, 0.90 mg/1 TRB, 1.60 mg/1 TRB and a reference concentration of 0.00 mg/1 TRB. Observations of mortality for both grass shrimp and blue crabs and change in body color from transparent to an opaque (sublethal effect) condition for grass shrimp were made at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours during the first 24-hour period and every 12 hours thereafter for the remainder of the 96-hour study. Observations during the dark phase of the photoperiod were made with the aid of a flashlight which had a red cellophane cover over the lens to reduce sensory stimulation. The criteria for determining death were the cessation of appendage movement and scaphognathite activity. Each species was isolated from each other as well as the individuals of each species during an experiment.

Total residual bromine chloride (TRB) concentrations were measured in each test aquarium at the same frequency as the mortality observations by amperometric titration (Fisher and Porter amperometric titrator, Model No. 17T1010, Fisher and Porter Co., Warminster, PA) procedures outlined for chlorine in *Standard Methods*. Equivalent total residual chlorine concentrations were converted to TRB concentration by multiplying by the factor 1.6 (molecular weight BrCl ÷ molecular weight Cl_2).

The bromine chloride bioassay system was supplied by a continuous flow of Patuxent River water which was split in the laboratory to supply: 1) five experimental and one reference test aquaria at 1.0 (± 0.13) liter/minute per aquarium which had a total volume of 2 liters and 2) a head box for making a bromochlorinated stock solution (approximately 40 mg/1 TRB).

Laboratory grade bromine chloride (Dow Chemical USA, Midland, MI) was vaporized in a Capitol Control gas evaporator (Capitol Control Co., Colmar, PA), metered to a venturi activated Capitol Control ejector system and mixed with river water before it entered the stock head box. The stock solution was then metered at a specific volume for each experimental concentration and mixed with river water before entering each experimental aquarium.

All toxicity data were evaluated statistically from both a concentration and response time standpoint. The effects of concentration on mortality (LC50) to grass shrimp and blue crabs and change of color (ET50) in grass shrimp were determined by LITCHFIELD and WILCOXON's (1949) method of log-probit transformations for concentration effect curves. The median time to death (LT50) of grass shrimp and blue crabs and median time to color change (ET50) of grass shrimp at specific concentrations of TRB were established by LITCHFIELD's (1949) modification of BLISS' (1937) method of log-probit transformations for time effect curves. All statistical tests were performed at the 95 percent significance level.

RESULTS AND DISCUSSION

Grass shrimp 24-h, 48-h and 96-h LC50's were 1.1, 0.8 and 0.6 mg/l TRB, respectively (Table 1). All LC50 values were significantly different. No significant difference was found between the 48-h and 96-h EC50 values of 0.4 and 0.5 mg/l TRB for grass shrimp. A significant difference was found between the the 48-h EC50 and 48-h LC50 and 96-h EC50 and 96-h LC50 of grass shrimp. Thus it can be seen that the sublethal color change response preceded death in both cases. This suggests that concentrations considerably lower than those obtained for the 48-h and 96-h LC50's may have an adverse effect on grass shrimp between 48 and 96 hours.

ROBERTS (pers. commun., 1977) has studied the acute toxicity of TRB to the same species of grass shrimp used in this study. His experimental conditions were similar to those used in this study, that is, continuous exposure to TRB, similar ambient water temperatures, etc. However, the salinity used by ROBERTS was approximately 2.5-fold higher (18-20 $^{o}/oo$) than that used in the present study. The 48-h and 96-h LC50's obtained by ROBERTS were 0.8 and 0.7 mg/l TRB, respectively. The values are almost identical to those obtained in this study. Since the grass shrimp is a euryhaline organism, this implies that bromine chloride residuals may be similar at both salinities. In contrast to bromine chloride, it has recently been suggested that the toxicological effects of chlorine may be a salinity dependent factor because chlorine residuals may change with salinity (BLOCK *et al.*, MS in press).

TABLE 1

Grass shrimp LC50's, blue crab LC50's, grass shrimp EC50's and associated log-probit statistics for each curve.[a]

Animal	Parameter	Median Concentration (± S.E.eq.)	Median Concentration 95% Confidence Limits	Slope Function (S)	Slope Function 95% Confidence Limits
Shrimp	24-h LC50	1.1(±1.19)	0.92 - 1.60	1.33	1.12 - 1.58
	48-h LC50	0.8(±1.23)	0.65 - 0.97	1.39	1.11 - 1.74
	96-h LC50	0.6(±1.23)	0.44 - 0.67	1.39	1.19 - 1.62
Crab	48-h LC50	1.2(±1.43)	1.11 - 2.85	1.78	0.81 - 1.64
	96-h LC50	0.8(±1.42)	0.58 - 1.16	1.76	1.11 - 1.77
Shrimp	48-h EC50	0.5(±1.22)	0.42 - 0.62	1.38	1.10 - 1.72
	96-h EC50	0.4(±1.22)	0.36 - 0.54	1.38	1.10 - 1.73

[a]Concentration reported in mg/1 total residual bromine chloride.

Significant differences ($P<0.05$) were found between the 48-h and 96-h LC50 values for blue crabs (Table 1). The 48-h LC50 was 1.2 mg/l TRB; the 96-h LC50 was 0.8 mg/l TRB. In a preliminary study only, ROBERTS (pers. commun., 1977) has found under the conditions described above a 96-h LC50 of approximately 1.0 mg/l TRB for non-molt mature blue crabs (7.6-10.2 cm wide) and a 96-h LC50 of approximately 0.6 mg/l for non-molt juvenile blue crabs (2.5 cm wide). The 96-h LC50 obtained in this study for non-molt juvenile blue crabs (2.6 cm wide) is similar to that obtained by ROBERTS. As in the case of grass shrimp, it appears that blue crabs may have similar LC50's at different salinities. ROBERTS' data also suggest that size may play a role in acute bromine chloride toxicity.

A comparison of sensitivities between grass shrimp and blue crabs at 48 and 96 hours was attempted, however, the test for parallelism (LITCHFIELD and WILCOXON, 1949) showed that the curves between species at both 48 and 96 hours were not parallel. Thus a definite comparison between LC50's for the two species could not be made even though they were studied under identical conditions (BROWN, 1973). Sufficient concentration data were not available to plot toxicity curves for the two species which may have permitted a comparison in terms of the incipient LC50 for each species (SPRAGUE, 1970).

The quantal response parameters of time to death (LT50) for grass shrimp and blue crabs and time to color change (ET50) for grass shrimp at fixed concentrations were also used to assess the acute toxicity of TRB. The LT50's for grass shrimp and blue crabs at 1.60 mg/l TRB were 15.3 and 36.5 hours, respectively (Table 2). A LT50 of 41.0 hours was established for grass shrimp at 0.90 mg/l TRB. A LT50 for blue crabs at 0.90 mg/l was not determined because 50% of the test animals were still living at 96 hours. A comparison of the LT50 curves for grass shrimp at 0.90 and 1.60 mg/l TRB revealed that the two curves were not parallel, therefore, statistical differences between curves could not be established. The ET50's for rate of color change in grass shrimp at 0.50, 0.90 and 1.60 mg/l TRB were 39.0, 6.6, and 3.6 hours, respectively. None of the ET50 curves were parallel, hence statistical differences between the curves could not be determined by log-probit statistics. As was the case for concentration effects, sufficient data were not available to plot time response toxicity curves for the two species.

Non-parallelism of curves within a species for a particular response parameter at different concentrations of a toxicant has been interpreted by some investigators as an indication that different modes of toxicity might be occurring (SPRAGUE, 1969). We do not feel we can speculate about the possible modes of TRB toxicity because the toxicological mechanisms of strong oxidizing agents to aquatic organisms are very poorly understood at the present time (BLOCK *et al.*, MS in press). Likewise, it is

TABLE 2

Grass shrimp LT50's, blue crab LT50's, grass shrimp ET50's and associated log-probit statistics for each curve.[a]

Animal	Parameter	Median Time (± S.E.eq.)	Median Time 95% Confidence Limits	Slope Function (S)	Slope Function 95% Confidence Limits
Shrimp	0.90 mg/l LT50	41.0(±1.30)	31.49 - 53.38	1.83	1.77 - 1.88
	1.60 mg/l LT50	15.3(±1.18)	12.97 - 17.93	1.45	1.42 - 1.48
Crab	1.60 mg/l LT50	36.5(±1.50)	24.37 - 54.68	1.92	1.80 - 2.05
Shrimp	0.50 mg/l ET50	39.0(±1.86)	20.96 - 72.58	4.13	3.84 - 4.43
	0.90 mg/l ET50	6.6(±1.54)	4.29 - 10.16	2.67	2.54 - 2.81
	1.60 mg/l ET50	3.6(±1.06)	3.35 - 3.76	1.14	1.13 - 1.15

[a]Time reported in hours.

difficult to speculate about different mechanisms influencing color change rates especially when one considers the fact that change of color in grass shrimp may be a general stress syndrome response.

In conclusion, it appears that there are differences in sensitivities between species when they are continuously exposed to TRB. Similar observations have been made for several estuarine organisms exposed to total residual chlorine (ROBERTS *et al.*, 1975). Size and age within species may play a role in TRB toxicity. This has been documented for total residual chlorine. In contrast to chlorine, salinity may not affect the toxicity of bromine chloride. It appears from ROBERTS' *et al.* (1975) chlorine data and the data discussed in this report, that grass shrimp may be more sensitive to total residual chlorine than TRB. Further comparative studies are needed, however, to resolve this point. More research is also needed to establish the potential differences and similarities in toxicity (if any) between the two halogens before bromine chloride can be accepted as an environmentally more desirable disinfection and antifouling agent than chlorine.

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