EFFECTS ON CARP EMBYROS (CYPRINUS CARPIO) AND DAPHNIA PULEX OF CHLORINATED ORGANIC COMPOUNDS PRODUCED DURING CONTROL OF FOULING ORGANISMS[1,2]

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DISCLAIMER

INTRODUCTION

Chlorinated organic compounds have been identified in cooling, waste treatment, and drinking waters after application of chlorine.[1] Concern for the potential public health and environmental impact of these compounds, as expressed at the First Conference on Water Chlorination,[2] was, in part, exacerbated by results reported at the Second Conference in 1977.[3] The advisability of chlorination for some purposes and the suitability of present chlorination technology for others have been questioned.[4-7] Because answers to these questions have long-term implications for public health and environmental quality, a strong empirical data base is desirable.

Cooling waters, by reason of volume alone, are a potentially significant source of chlorinated organics of which twenty-one have already been identified.[8,9] Although Trabalka and Burch[1,10] determined the acute toxicity of two synthetic power plant effluents and many of the 17 component compounds to carp embryos (Cyprinus carpio) and Daphnia pulex, more complete studies are needed. For example, information on environmental persistence and fate, acute and chronic toxicity, and bioaccumulation of power plant chlorination products is needed to assess their potential for environmental impact.

In this paper, we report results of: (1) decay studies of the trihalomethanes and four nonvolatile chlorinated organics; (2) acute toxicity tests with four trihalomethanes and carp embryos; and (3) chronic toxicity tests with Daphnia pulex and selected chlorinated organics and synthetic effluents under limited conditions.

MATERIALS AND METHODS

Acute toxicity of four trihalomethanes identified in cooling waters[9] was determined using the carp embryo (Cyprinus carpio) test procedure described previously.[1] Toxicant solutions were renewed at 8-hr intervals. Percent survival of eggs at hatching was used to calculate a 4- to 5-day LC_{50}.

Decline in toxicant concentration during the period between solution changes in the tests was monitored by gas chromatographic (GC) analysis of samples of control test containers without eggs. Stock solutions of trihalomethanes were diluted with distilled water to concentrations near the 96-hr LC_{50} for carp embryos. These solutions were then added to 300-ml glass containers (fitted with plastic covers with a single 64-mm hole) and incubated at 26.5 1 C under a 14-hr-light - 10-hr-dark cycle. Illumination at the water surface was 807.2 Lumen/m^2. Triplicate containers were sampled after 0, 1, 2, 4, and 8 hr for GC analysis. Stock solutions of four nonvolatile compounds, at the lowest concentrations at which deleterious effects on Daphnia pulex were observed, were incubated under test conditions. Triplicate containers were sampled after 0, 24, 48, and 96 hr. Impurities were removed and the sample concentrated for GC analysis. All compounds were analyzed by GC using a Flame Ionization Detector. A glass column with Tenax™ packing was used for analysis of the trihalomethanes and 8-chlorocaffeine. A glass column with Carbopac™/0.1% sp. 1000 packing was used for analysis of the phenols. More complete information on methodology will be presented elsewhere in another publication.

Chronic toxicity tests were done with the cladoceran Daphnia pulex and two synthetic effluents and their constituents using the methods described previously.[1] Test concentrations in decimal increments ranged from 0.01 to 100 mg/liter. The chloro-organic composition (17 nonvolatile compounds) was based on identifications from power plant cooling waters reported by Jolley et al.[1,8] Synthetic mixtures were prepared from laboratory stocks of the individual compounds. The halogenated organics used in the chronic exposure tests were either those identified as most toxic in acute toxicity tests[1] (resorcinol, 4-chlororesorcinol, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, 4-chloro- 3-methylphenol), or those known to be present at highest concentrations in actual chlorinated effluents (4-chloro-phenylacetic and 5-chloro-salicylic acids, 5-chlorouracil, and bromoform - representing the haloform group). The indices of chronic toxicity criteria investigated were maturation rate (as indicated by date of first reproduction), reproductive rate (adjusted for mortality), and shortening of life span.

RESULTS AND DISCUSSION

Decline in concentration of the trihalomethanes was substantially faster than that of the nonvolatile chorinated organics. The trihalomethanes disappeared rapidly despite the use of a semi-enclosed container. Half-lives ranged between 5.0 and 7.2 hr (Table 1). Average concentrations during the 8-hr period between toxicant renewals ranged from 39 to 68% of the initial concentration. In contrast, the nonvolatile chlorinated organics had half-lives of more than 55 hr in

the test medium (Table 1). Average concentrations during the 3.5-day renewal period were between 62 to 94% of the initial concentration.

Dibromochloromethane was the most toxic and chloroform the least toxic of the trihalomethanes to carp embryos (Table 2). Apparently, toxicity is not related to the obvious structural or physical characteristics of the four compounds.

Since the objective of the tests was to indicate potential toxicity of cooling waters and because the LC_{50} values are already a minimum of three orders of magnitude greater than the highest effluent concentrations reported, we did not correct the LC_{50} values for the decline in toxicant concentration between solution renewals (factor of 2-3).

The results of acute toxicity tests with Daphnia pulex, two synthetic chlorinated effluents, and individual compounds, were published earlier[1] and are summarized here for comparison with the results of the present study. Acute toxicity of the two synthetic effluents occurred at concentrations approximately three orders of magnitude greater than those reported in actual effluents, and for individual compounds of which resorcinols were the most toxic, the concentrations were 2 to 4 orders of magnitude greater than those in actual effluents. Toxic contributions from individual compounds to toxicity of the synthetic effluents were less than additive, i.e., 96-hr LC_{50} values were 2.1 to 2.6 times greater than expected from simple additivity.

Results of the chronic exposure tests, summarized in Fig. 1, are not substantially different from the general pattern observed in acute

tests, although detrimental effects are observed at lower concentrations in the former tests, as expected. Individual compounds caused significant decreases in life span at concentrations approximately 1 to 4 orders of magnitude greater than those reported in actual chlorinated effluents, and decreases in reproductive rate occurred at concentrations approximately 2 to 4 orders of magnitude greater than in actual effluents. As in the acute tests, resorcinols were the most toxic of the individual compounds tested. The two synthetic effluents produced significant decreases in life span and reproductive rate at concentrations approximately 2 orders of magnitude greater than those reported from chlorinated effluents.

Although a complete test of additivity test cannot be done on incomplete data from chronic exposure tests, response patterns suggest that the sum of toxic contributions of individual compounds to the total toxicity of the synthetic effluents should be additive or (more probably) less than additive. Most compounds produced detrimental effects at concentrations 2 orders of magnitude above those reported from actual effluents. For effects on reproductive rate, the sum should be less than additive since all organics tested met this criterion. Performance of an additivity test for chronic exposure effects is further complicated by the appearance of apparent stimulatory responses (statistically significant increases in life span, reproductive rate, or maturation rate compared with those of controls), generally at concentrations just below the threshold concentration for deleterious effects. This phenomenon occurred with most of the materials we tested (Fig. 1).

The relative maturation rates (as determined by onset of reproduction) and total reproduction (average total number of offspring produced during a lifetime) for D. pulex in response to all test materials were not included in Fig. 1, because they were less sensitive than either shortening of the life span or reproductive rate as chronic toxicity criteria. Onset of reproduction was not delayed in cases where life span and reproductive rate were not also significantly reduced. In only one test were significant decreases in life span and reproductive rate accompanied by a significant decrease in maturation rate. However, in all cases a decline in total reproduction was accompanied by a significant decrease in either life span or reproductive rate.

CONCLUSIONS

Although our studies of the chemical half-lives of halogenated organics were not designed to mimic field conditions, they do permit qualified predictions. Dilling et al.[11] calculated the half-life of chloroform as 21 min in a rapidly mixed system. Under our conditions the half-life for chloroform was 5.2 hr. The lower value is probably a better estimate for surface discharge conditions, but the actual value will be affected by both the depth and mixing rate of the entire water column. However, the volatile trihalomethanes will probably not persist for extended periods in power plant discharge waters. Nonvolatile organics should disappear less rapidly than volatile compounds from effluent waters and, therefore, they may be transported over greater distances. However, other factors may also be important

in determining the persistence of less volatile compounds: sorption, microbial degradation, hydrolysis, etc. Light intensity (photolysis) and pH are also important in the degradation of organics. For example, Southworth and Gehrs[12] reported that 5-chlorouracil decomposed under UV light 17 times faster at pH 8.6 than at pH 7.3.

The intermittent chlorination schedule of most power plants at freshwater sites may reduce the potential acute and chronic effects of the organics we tested, unless they are accumulated to significant levels by resident biota (unlikely because of high water-solubility). However, further study is needed on the fate of these compounds in the environment before their hazards can be assessed. In particular, information on their degradation and uptake by aquatic organisms and on their removal by physicochemical mechanisms under natural conditions is needed.

We conclude that both the acute and chronic toxicities to D. pulex of the synthetic chlorinated effluents we tested were primarily attributable to chlorophenols. Chronic toxicity was demonstrable at concentrations an order of magnitude lower than those required to produce acute toxicity, but was still observed at concentrations 10 mg/liter, which are two orders of magnitude greater than concentrations reported from actual effluents.

The most sensitive indicators of chronic toxicity in our test regime were effects on reproductive rate and shortening of life span, perhaps because these characteristics reflected cumulative effects of long-term exposure to a toxic agent. Maturation rate apparently is a less sensitive indicator because the exposure time to first reproduction is only a small fraction of the life cycle.

The phenomenon of stimulation of reproduction or increase in length of life span just below concentrations producing deleterious effects merits further investigation for several reasons. First, this phenomenon may partly explain why less than additive toxicity often appears when toxic contributions from constituents of a mixture are summed. Second, although the response appears beneficial to the test organism, the cause may actually be related to toxicity of the organics to the microorganisms of the test system. Selective inhibition of these constituents could result in a direct benefit to the test organism by reduction of pathogens, or indirectly, by removing competitors that normally reduce concentrations of a valuable food organism . Thus, the explanation for this phenomenon may be extremely valuable in assessment of environment-mediated exposures of aquatic organisms to complex effluents.

Final determination of the impacts of chlorinated organics on aquatic ecosystems is not possible. Barring awareness of aquatic species with substantially greater sensitivity than the ones we used and/or knowledge of significant bioaccumulation by aquatic organisms, the chlorinated organics we have examined do not appear to be of particular environmental concern. However, most of the chlorinated organic fractions of the two power plant effluents studied remain to be identified. Some of these compounds may prove to be more toxic than those we have reported here. Under our present test regime, each of these compounds would require time-consuming identification and toxicity testing.

We recommend that efforts be increased to develop techniques for producing concentrates of actual chlorinated effluents. This would permit preliminary screening of a wide variety of effluents, with extensive examination limited to those that demonstrate biological activity. Although this approach is not without risk, progress would be enhanced and extensive efforts yielding "no effect" determinations (with all the necessary qualifications) such as those we have presented here will be reduced.

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Table 1. Decline in concentration of halogenated organics in test media during renewal intervals

Compound	Initial concentration (mg/liter)	Half-life (hr)	Fractional aver. conc. during renewal period[a]
Trihalomethanes			
Chloroform	160	5.0	0.57
Dichlorobromomethane	10	2.9	0.39
Chlorodibromomethane	50	5.7	0.62
Bromoform	75	7.2	0.68
Nonvolatile organics			
4-chlororesorcinol	0.1	64	0.62
4-chlorophenol	1.0	56	0.64
4-chloro-3-methylphenol	1.0	96	0.84
8-chlorocaffeine	10	96	0.92
5-chlorouracil	2	504	0.93[b]

[a]Solutions were changed every 8 hr for trihalomethanes and every 3.5 days for nonvolatile compounds. Average concentration expressed as a fraction of the initial concentration.

[b]Based on G. R. Southworth and C. W. Gehrs, Water Res. 10:967-971 (1976).

Table 2. Toxicity of trihalomethanes to carp embryos (Cyprinus carpio) exposed from fertilization to hatching

	Compound[a]			
	Chloroform	Bromo-dichloromethane	Dibromo-chloromethane	Bromoform
LC_{50}	160	119	53	76
95% C.L.	157-165	111-137	52-54	74-79

[a]Nominal concentrations in mg/liter.

LIST OF FIGURES