Bull. Environ. Contam. Toxicol. (1989) 42:315–319

Bulletin of Environmental Contamination and Toxicology

Toxicity of Chlorine Dioxide to Early Life Stages of Marine Organisms

Jo Ellen Hose,[1] Dean Di Fiore,[1] Heidi S. Parker,[1] and Terry Sciarrotta[2]

[1]VANTUNA Research Group, Department of Biology, Occidental College, 1600 Campus Road, Los Angeles, California 90041, USA and [2]Southern California Edison Company, Environmental Research, P.O. Box 700, Rosemead, California 91770, USA

With increasing interest in minimizing exposure to chlorine, many electric generating and water treatment plants are exploring the use of alternative biocides such as chlorine dioxide (Sussman and Ward 1977; EPA 1980). Unlike chlorine, chlorine dioxide does not react with ambient organic compounds to form potentially carcinogenic trihalomethanes such as chloroform. After chlorine dioxide treatment, stable haloamines are not produced and compounds containing aromatic rings are fully oxidized (EPRI 1980). However, the toxicity of chlorine dioxide to aquatic organisms has received little study. Using a continuous exposure regimen typical of East Coast electric generating stations, chlorine dioxide was found to be two to four times more toxic than total residual chlorine (TRC) to freshwater fish (Wilde et al. 1983).

No information exists on chlorine toxicity to marine organisms. Furthermore, West Coast electric power stations usually discharge chlorine intermittently once or twice daily and substantial mixing of receiving water occurs between treatments. Therefore, this study sought to obtain information on chlorine dioxide toxicity using an exposure schedule typical of generating stations which discharge into the marine environment. Early life history stages of a plant, invertebrate and fish were tested since these stages are generally acknowledged to be most sensitive to toxicants (McKim 1977) and are the stages that are most likely to be exposed to the effluent.

MATERIALS AND METHODS

Sublethal measurements of chlorine dioxide toxicity (germination success and vegetative growth) were based upon a recent bioassay protocol (Anderson and Hunt, in press). Reproductive blades (sporophylls) of giant kelp (Macrocystis pyrifera) were rinsed with filtered (5 um) seawater, and stored overnight at 5°C. Sporophylls were dessicated for 1 h, then immersed in filtered seawater for 2 h to release the meiospores. Four milliliters of a meiospore solution (total of 450,000 meiospores) were inoculated into each of five replicate beakers already containing glass microscope slides and 500 mL filtered seawater or toxicant. Dilutions were prepared using a stabilized aqueous solution of 25% chlorine dioxide (Rio Linda Chemical Co., Rio Linda, California) and ranged from 2.5 ug/L to 250 mg/L. This range in concentration brackets the predicted exposure levels that would be experienced in the environment. Meiospores were incubated

Send reprint requests to Dr. Hose at the above address

for 48 h at 15°C with continuous illumination provided by cool-white fluorescent lights (100 uE/m^2/sec). Glass slides were removed and 100 spores were examined using a light microscope for evidence of germination. Ten random measurements of germ tube length were performed for each replicate using an ocular micrometer at 400X magnification.

The 48-h purple sea urchin (Strongylocentrotus purpuratus) embryo bioassay was performed using methods similar to those of Oshida et al. (1977). Gametes were collected following intracoelomic injection of 0.5 M KCl and an approximate sperm:egg ratio of 1500:1 was used for fertilization. Fifteen minutes following fertilization, 15,000 embryos were inoculated into five replicate 600 mL beakers for each concentration (2.5 ug/L to 250 mg/L chlorine dioxide) and control. Embryos were incubated for 48 h at 15°C. At 48 h, a 10-mL sample from each bioassay beaker was placed in a vial and fixed with 3 mL of buffered formalin. A 1-mL aliquot of sample was placed in a Sedgewick-Rafter counting chamber for determination of abnormalities. Duplicate counts were performed on each sample and abnormalities were grouped into one of five categories: pre-hatch malformations, retarded development, post-hatch malformations, skeletal malformations and gut malformations.

Kelp bass (Serranidae: Paralabrax clathratus) were spawned using 50 ug/kg of luteinizing hormone-releasing hormone analog (Sigma). Eggs were collected and fertilized with stripped milt. They were allowed to settle for 4 h and the floating viable eggs rinsed several times with filtered seawater. Eggs were maintained at 20°C without aeration. Bioassay procedures were those of Cross et al. (1987) except that 5 chlorine dioxide concentrations (2.5 ug/L to 25 mg/L) were tested in addition to the control. Twenty-hour old eggs were stocked into 5 replicate culture dishes containing 30 mL of solution. Plastic covers were placed 6 cm above the dishes to keep out dust particles. Bioassay temperature was 20°C. Hatching occurred 40 h post fertilization. After 48 h exposure, dead eggs and larvae and surviving larvae were counted using a dissecting microscope.

Water quality parameters (salinity, temperature, dissolved oxygen and pH) were monitored at the termination of each bioassay and were within limits required by APHA (1980). Salinity was 33 ppt.

Statistical differences between endpoints were determined using analysis of variance followed by the Student-Newman-Keuls multiple range test ($p=0.05$). Percentages were first transformed using $y=\log(x+1)$.

RESULTS AND DISCUSSION

A single exposure of chlorine dioxide produced effects in early life stages of marine organisms only at high doses. Germination of Macrocystis pyrifera was significantly reduced at chlorine dioxide concentrations of 25 and 250 mg/L (Table 1). At the highest dose, germination was decreased by about 80%. Germ tube length was significantly reduced only at 250 mg/L. Developmental abnormalities in the sea urchin were evident only at the highest concentration, 250 mg/L. Compared with the control, pre-hatch malformations were 6% higher; retarded development, 2%; post-hatch malformations, 20%; skeletal malformations, 21%; and gut malformations, 11%. These data suggest that although chlorine

Table 1. Effects of chlorine dioxide on early life stages of giant kelp (Macrocystis pyrifera), purple sea urchin (Strongylocentrotus purpuratus), and kelp bass (Paralabrax clathratus). Concentrations which are significantly different from the control are starred.

Organism	Endpoint	Dose mg/L	Response X + SD (n)
Kelp	% Germination	0.0000	38.2 ± 7.5 (5)
		0.0025	48.2 ±11.4 (5)
		0.025	38.0 ± 6.7 (5)
		0.250	30.2 ± 6.9 (5)
		2.50	31.6 ± 9.3 (5)
		25.0	21.2 ± 5.3 (5)*
		250.0	8.0 ± 1.5 (5)*
	Germ Tube Length	0.0000	6.4 ± 0.5 (5)
		0.0025	5.7 ± 1.0 (5)
		0.025	5.9 ± 0.9 (5)
		0.250	5.6 ± 0.5 (5)
		2.50	6.9 ± 0.3 (5)
		25.0	5.9 ± 1.2 (5)
		250.0	3.9 ± 3.9 (5)*
Urchin	% Abnormal	0.0000	9.3 ± 3.4 (5)
		0.0025	10.5 ± 3.6 (5)
		0.025	9.3 ± 2.1 (5)
		0.250	15.1 ± 4.4 (5)
		2.50	10.7 ± 3.5 (5)
		25.0	12.0 ± 2.3 (5)
		250.0	15.1 ± 2.1 (5)*
Fish	% Survival	0.0000	78.0 ± 7.5 (5)
		0.0025	78.0 ± 14.0 (5)
		0.025	79.0 ± 12.5 (5)
		0.250	88.0 ± 7.5 (5)
		2.50	87.0 ± 11.5 (5)
		25.0	82.0 ± 17.5 (5)

dioxide is rapidly degraded, there remains a delayed effect on differentiating (post-hatch) embryos. Survival of larval kelp bass (Paralabrax clathratus) was not significantly affected by chlorine dioxide.

Relative sensitivities of early life stages of these marine organisms to single doses of chlorine and chlorine dioxide were similar although the latter biocide was markedly less toxic. No effect concentrations (NOECs; EPA 1985) were roughly a thousand times higher for chlorine dioxide than for total residual chlorine (TRC; unpublished results). Using a similar exposure method but assessing a different endpoint (sexual reproduction

versus asexual reproduction assayed in the present study), production of sporophytes was significantly reduced at a mean TRC concentration of 58 ug/L. The mean NOEC for sea urchin malformations was 86 ug/L TRC while survival of kelp bass larvae was not significantly affected at the highest dose tested, 436 ug/L. However, the NOEC for larvae of another indigenous southern California fish species (white croaker, Genyonemus lineatus) was 337 ug/L TRC.

Results obtained here using a single chlorine dioxide exposure typical of a once-through steam generating plant discharging into marine waters apparently underestimate the toxicity resulting from continuous exposure. Mean 96-h LC50 values for juvenile fathead minnows (Pimephales promelas) were 20 ug/L chlorine dioxide, 170 ug/L for adult fathead minnows, and 150 ug/L for young-of-the-year bluegills (Lepomis macrochirus; Wilde et al. 1983). Chlorine dioxide selectively attacks chlorophyll molecules and oxidizes hemoglobin (EPRI 1980), and chronic exposure (9 mg/L per day) was shown to suppress thyroid hormone metabolism in monkeys (Bercz et al. 1982). Thus, minimal toxicity is predicted by the intermittent discharge of chlorine dioxide into receiving waters which undergo rapid mixing compared to situations with static receiving waters or continuous discharge of chlorine dioxide.

Acknowledgments. We thank N. Macalinao and S. Bay for their technical assistance and W. Westphal for statistical advice. The project was funded by Southern California Edison Company under contract C3006906.

REFERENCES

Anderson BS, Hunt JW (in press) Bioassay methods for evaluating the toxicity of heavy metals, biocides and sewage effluent using microscopic stages of giant kelp Macrocystis pyrifera (Agardh): A preliminary report. Mar Environ Res

APHA (American Public Health Association). 1980. Standard methods for the examination of water and wastewater. 15th ed. Washington D.C.

Bercz JP, Jones L, Garner L, Murray D, Ludwig DA, Boston J (1982) Subchronic toxicity of chlorine dioxide and related compounds in drinking water in the nonhuman primate. Environ Health Persp 46:47-55

Cross JN, Hardy JT, Hose JE, Hershelman GP, Antrim LD, Gossett, RW, Crecelius EA (1987) Contaminant concentrations and toxicity of sea-surface microlayer near Los Angeles, California. Mar Environ Res 23:307-323

EPA (Environmental Protection Agency) (1980) Effluent limitations, guidelines, pretreatment standards under Clean Water Act; steam electric power generating point source category, proposed regulations, 45 F.R. 68328.

EPA (Environmental Protection Agency) (1985) Methods for measuring acute toxicity of effluents to freshwater and marine organisms, 3rd ed. EPA/600/4-85-013. USEPA, Cincinnati, Ohio. 216 pp.

EPRI (Electric Power Research Institute) (1980) Review of open literature on effects of chlorine on aquatic organisms. EPRI EA-1491. Palo Alto, California.

McKim JM (1977) Evaluation of tests with the early life stages of fish for predicting long-term toxicity. J Fish Res Board Can 34:1148-1154.

Oshida, PS, Goochey TK, Mearns AJ (1981) Effects of municipal wastewater on fertilization, survival and development of the sea urchin, Strongylocentrotus purpuratus, In: Vernberg FJ, Calabrese A, Vernberg WB, (eds) Biological monitoring of

marine pollutants. Academic Press, New York. p 389
Sussman S, Ward WJ (1977) Microbiological control with chlorine dioxide helps save energy. Mat Perform 16:24-28.
Wilde EW, Soracco RJ, Mayack LA, Shealy RL, Broadwell TL, Steffen RF (1983) Comparison of chlorine and chlorine dioxide toxicity to fathead minnows and bluegill. Water Res 17:1327-1331.

Received January 25, 1988; accepted June 13, 1988.