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Toxicity Studies of Antiscalant Agents Using *Arbacia punctulata* Gametes and Embryos as Test Organisms

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Various substances are used industrially to inhibit scaling or mineral deposition in cooling towers, boilers and other devices. Crystal formation in industrial boilers and cooling towers promotes corrosion and reduces heat exchange (Nancollas 1987; Seels 1987). Because cooling towers utilize freshwater sources such as lakes or streams to cool machinery by recirculation, eventually water treatment chemicals used to control scaling and corrosion are released to the environment.

Typical antiscaling compounds include polyacrylate (Rohm and Haas 1985), and polyacrylamide based polymers (Nalco 1987). These commercial antiscalants are of concern because they are used extensively in flow-through systems. Industrial effluents and municipal wastewaters contain antiscalants that are introduced into aquatic systems such as lakes, streams and estuaries. The toxicity of these water treatment polymers is important considering the trillions of gallons of water used annually by industry (Brooks 1986). In addition to this, many water treatment chemicals such as polyacrylamide and polyacrylate based polymers are not biodegradable (Kapoor et al. 1986).

Organic matrix proteins regulate the crystallization of calcium carbonate in many invertebrates, and calcium phosphate in vertebrate biominerals (Weiner et al. 1983; Veis 1989). Oyster shell matrix and its polyanionic polyamino acid analogs have been evaluated for use as antiscalants and corrosion inhibitors (Little and Sikes 1991) in water treatment. Polyaspartate, which is the simplest example of this class of compounds has been shown to be an excellent inhibitor of crystallization *in vitro* (Wheeler et al. 1988).

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In this study, the commercial water treatment chemicals, polyacrylate and a polyacrylamide based polymer (polyacrylamide), were evaluated for toxic effects using sea urchin (Arbacia punctulata) gametes and embryos as test systems. These results were compared to those obtained with thermally polymerized polyaspartate, a possible alternative water treatment polymer.

MATERIALS AND METHODS

From September to April, specimens of Arbacia punctulata were collected along the Florida Gulf Coast or ordered from Gulf Coast Specimens, Panacea, Florida. Gametes were obtained using the procedures of Tyler, (1949). Collected eggs were washed with sea water three times by settling and rinsing, to remove substances secreted by the female, which may act as a block to fertilization (Hinegardner 1975).

Polyacrylate of 2100 MW was purchased as a sodium salt from Polysciences, Inc. Polyacrylamide based "prism" polymers of 5000-8000 MW were obtained from Nalco Chemical Company. These prism polymers are terpolymers composed of acrylate, acrylamide and sulfonated acrylamide (Nalco 1987). Both the polyacrylate and polyacrylamide based polymers were used in bulk form, as supplied by the manufacturer, and in purified form. Polymers were purified by dialysis for 4 hours against distilled water (1000 MW cutoff Spectra/Por dialysis tubing) and lyophilized. Polyaspartate (thermal polyaspartate) was polymerized by thermal polycondensation, in which aspartic acid was placed in a two liter evaporator vessel emersed in cooking oil at 190°C ± 2°C for 96 hours (Little and Sikes 1991).

Following thermal polymerization, the polymer of aspartic acid is primarily in the polyanhydro or aspartimide form. Polyanhydro-aspartic acid was converted to polyaspartate, its more active form, by mild alkaline hydrolysis (Little and Sikes 1991).

Effects of the polymers on sperm and embryo respiration were examined using a YSI s331 oxygen probe, attached to a probe adapter which supplied a voltage across the electrode (0.8v). The polymers were added to ASW at 0.1 mg/ml, 0.5 mg/ml and 1.0 mg/ml, for sperm respiration with the pH adjusted to match the control ASW (approximately 8.2). The polymers were added to ASW at 1.0 mg/ml, for embryo respiration (blastula/prism stage) with the pH also adjusted. The experimental vessel was a 10 ml glass vial that held the electrode and was fixed in position with an adhesive. A two to three milliliter solution of sea urchin sperm (10^8 sperm/ml) or embryos (10^4 embryos/ml) in ASW (or ASW with experimental

substance) was added to the vessel which was then placed in a constant temperature bath at 20° C. Respiration was measured for 20 minutes for each preparation of embryos or sperm. Decreases in microamperage that resulted from oxygen consumption were read using a multimeter connected to a chart recorder.

Effects of polymers on development were evaluated by exposure of embryos to these polymers at dosages of 0.1 and 1.0 mg/ml and monitoring growth through the pluteus stage. The experimental substances were added to ASW after fertilization for continuous exposure treatment. Sperm (100 ul) was added to a 400 ml solution of eggs (approximately six hundred eggs per ml). Fertilization success was observed after 20 minutes, and the sperm were removed by allowing the eggs to settle out and pouring off the sperm solution. Treated organisms were resuspended in 50 ml of ASW containing the specified polymer. Control organisms were resuspended in 50 ml ASW. Antibiotics (penicillium/streptomycin, Sigma Chemical) were added at 50 units/ml. In addition to continuous exposure, experimental substances were added to sea urchin cultures for a two hour pulse at early cleavage (30 minutes after fertilization), and blastula stage (15-20 hours after fertilization).

Cultures were monitored for development at blastula, prism and pluteus stages. The percentage of embryos reaching the blastula stage was determined by counting the number of free swimming individuals without fertilization membranes. The percentage of embryos reaching the prism stage was quantified by counting the number of swimming organisms with the characteristic pyramidal shape. The percentage of embryos developing into normal plutei was determined by counting the individuals with well formed spicular arms.

RESULTS AND DISCUSSION

Only sea urchin sperm treated with unpurified polyacrylamide based polymer at the levels tested showed a reduced respiration rate. This decrease was observed approximately 10 minutes after respiration measurement had begun (Figure 1) for both 0.5 and 1.0 mg/ml treatments. At 0.1 mg/ml, unpurified polyacrylamide based polymer caused no difference in respiration rates compared to control rate. Sperm treated with the polyacrylamide based polymer that was purified by dialysis did not affect respiration rates. Both purified and unpurified polyacrylate-treated sperm showed no difference in respiration rates between control and experimental treatments. Sperm treated with thermally polymerized polyaspartate also showed no change in respiration rates.

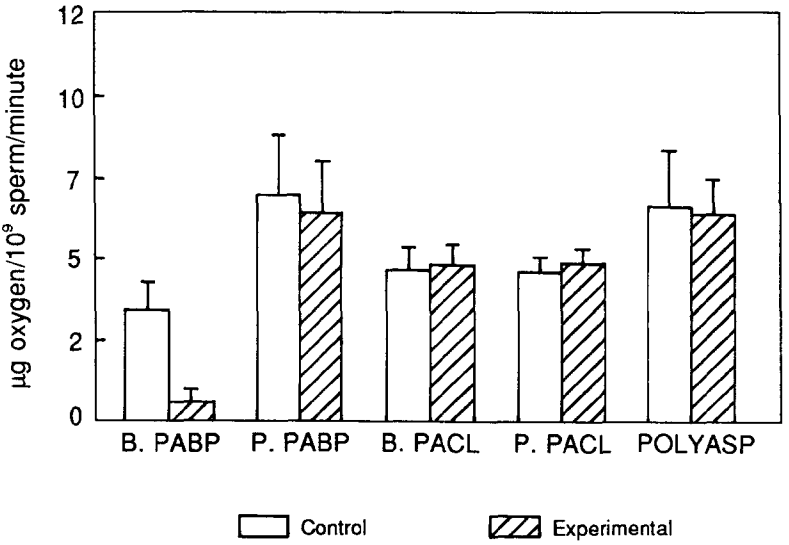

Figure 1. Respiration of sea urchin sperm treated with 1.0 mg/ml polymers. B. PABP represents bulk polyacrylamide-based polymer. P. PABP represents purified polyacrylamide-based polymer. B. PACL represents bulk polyacrylate. P. PACL represents purified polyacrylate. POLYASP represents thermal polyaspartate. Values are given as the mean respiration rate (n=9). Polymer was added at time zero.

Sea urchin embryo respiration decreased when embryos were treated with 1.0 mg/ml bulk polyacrylate. This concentration is greater than concentrations of water treatment polymers typically used industrially. A concentration of 1.0 mg/ml purified polyacrylate caused no change in respiration rates. Both purified and unpurified polyacrylamide treated embryos showed no difference in respiration rates. Embryos treated with thermally polymerized polyaspartate also showed no change in respiration rates.

Embryos treated with unpurified and purified polyacrylamide showed identical abnormalities when continuously exposed to the experimental substance (Table 1). These abnormalities include cessation of development at the morula (1.0 mg/ml) and blastula (0.1 mg/ml) stage. These types of effects have also been seen with high concentrations of metals and metabolic poisons in sea urchin embryos (Pagano et al. 1983; Kobayashi 1984). Embryos pulsed with purified polyacrylamide at the blastula stage also formed abnormal spicules or stopped development at the prism stage. This cessation of development at the prism stage is probably related to abnormal spicule formation since the major developmental

Table 1. Development of sea urchin embryos treated with experimental substances.

		EXPOSURE			
Polymer	dose mg/ml	continuous (n=6)	cleavage pulse (n=3)	blastula pulse (n=3)	blastula continuous (n=3)
Polyacrylamide based polymer	0.1	B (>90%)	N	P/AB (>50%)	P/AB (>90%)
Polyacrylate	1.0	P/AB (>99%)	N	P/AB (>50%)	P/AB (>99%)
Polyacrylate	0.1	N	--	--	--
Thermal Polyaspartate	1.0	P/AB (>99%)	N	N	P/AB (>99%)
Thermal Polyaspartate	0.1	N	--	--	--

B - Embryos stopped developing at blastula stage
P/AB - Embryos were at prism stage or pluteus stage with abnormal spicules
N - Normal development to pluteus
continuous - polymer added after fertilization
cleavage pulse - polymer added after fertilization for two hours
blastula pulse - polymer added during the blastula stage for two hours
blastula continuous - polymer added continuously from the blastula stage
(200 embryos counted per treatment).

step between prism and pluteus stages is spicule development. Since polyacrylamide in both the purified and unpurified forms caused developmental abnormalities in sea urchin embryos, the polymer exclusive of monomer contamination is responsible for the toxic effects. These effects are significant in terms of environmental contamination since 0.1 mg/ml polymer concentration is within the commercial treatment limits for cooling waters (Dubin 1991).

The major toxicological data on acrylates simply show them to be strong irritants (International Agency for Research on Cancer 1979). However, purified polyacrylate caused developmental defects in sea urchin embryos at a lower dosage (0.1 mg/ml) than unpurified polyacrylate. The reason for this is not obvious; however, one possibility is the effect of an increase concentration of higher molecular weight polyacrylates during dialysis. The developmental defects included abnormal spicules and a cessation of development at the prism stage (Table 1).

When continuously treated with polyaspartate at a concentration of 1.0 mg/ml, sea urchin embryos develop abnormal spicules and stopped development at the prism stage. This concentration of polyaspartate is beyond the range of polymer concentrations that would be used in cooling water systems. At a concentration 0.1 mg/ml, polyaspartate did not affect the development of sea urchin embryos. The effects seen with polyaspartate are probably due to the mineralization inhibiting properties of this substance which is an organic matrix analog. Organic matrix isolated from oyster shell has been shown to reduce spicule formation in sea urchin embryos without affecting general metabolism (Wheeler et al. 1988).

The findings of the present study are consistent with what is known about polyacrylamide based polymers. The primary toxicity of these polymers is due to acrylamide contamination, but the polymer alone has been shown to cause toxic effects in aquatic organisms (King and Noss 1989). Similar results were observed in this study where both purified and unpurified polyacrylamide based polymer caused cessation of development at the blastula stages at a concentration of 0.1 mg/ml. However the results of this study concerning polyacrylate, which is considered non-toxic (International Agency for Research on Cancer 1979), show it to affect sea urchin respiration (1.0 mg/ml) and development (0.1 mg/ml). The results of toxicity studies with polyaspartate, which show inhibition of spicule development, are consistent with studies of organic matrix effects on spicule development (Wheeler et al. 1988). However these levels are ten times greater than levels that would be used in an industrial setting. Compared to a polyacrylamide based

polymer and polyacrylate, two commonly used water treatment polymers, thermally polymerized polyaspartate is generally nontoxic.

An additional advantage of thermal polyaspartate is that it is enzymatically degradable (Cekolin 1991). Water treatment polymers such as polyacrylate and polyacrylamide based polymers are not biodegradable (Kapoor et al., 1986). In the aquatic environment, the persistence of a substance may be as important as its toxicity, since potential harm resulting from long term exposure is difficult to predict.

Thermal polyaspartate exhibits antiscaling properties comparable to these polymers (Sikes and Wheeler, 1985). It can be produced economically in large amounts by a thermal polymerization process (Little and Sikes 1991). As environmentally compatible polymers, polyaspartate and similar compounds offer favorable prospects for water treatment.

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