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TOXICITY OF A A CHLORINATED BENZENE TO SEA URCHIN EMBRYOS

The effects of chlorinated benzene compounds on marine animals are not well known. Until recently, only hexachlorobenzene (HCB) had received attention in pollution studies: In a report published by the National Academy of Sciences (1975) this substance is labelled a marine pollutant of special concern. Last year. Project scientists analyzed municipal wastewater samples for HCB and three other chlorobenzene compounds—para-dlchlorobenzene (p-DCB), ortho-dichlorobenzene (o-DCB) and 1,2,4-trichlorobenzene (1,2,4-TCB); the results indicated that the latter three substances were present at levels at least one order of magnitude higher than that of HCB (Young and Heesen 1976).

It has been shown that p-DCB can act as a mitotic poison, causing disorders of cell division that result in abnormal multinucleate cells (Biesele 1958). The study de-scribed here was a preliminary investigation of o-DCB's potential as a mitotic poison in the marine environment. Recently fertilized sea urchin eggs were exposed to an o-DCB concentration of about 21 mg/liter, and their development morphology and rate were observed during the subsequent 52 hours. The results showed that o-DCB has the potential to interfere with embryological development: Grossly abnormal sea urchin embryos were evident at about 28 hours past fertilization.

MATERIALS AND METHODS

The experiment involved two toxicant solutions. To form the first, o-DCB in liquid form was added directly to seawater; the o-DCB was introduced into the second solution via the aeration system. As this was a preliminary test of high concentrations of o-DCB, no replicate solutions were used. Precautions were taken to have as little bacterial growth in the test solutions as possible—all the seawater used in the experiment had been filtered through a 0.45-micron Millipore

filter and treated with streptomycin sulfate (50 pg/ml) and buffered penicillin 6 potassium (30 units/ml).

The first toxicant solution was made by adding 100 ml of o-DCB to 1,700 ml of seawater in a 2-liter glass jar. Because of the limited solubility of o-DCB in water and the fact that it is denser than seawater, two distinct liquid layers were visible in the jar—seawater at the top and the o-DCB on the bottom. When the experiment began, a small motor was activated to stir the solution continuously for the duration of the experiment. Air filtered "in-line" was pumped into the solution through a capillary pipette connected to the pump with aquarium air tubing (Figure 1).

The second toxicant solution involved 1,800 ml of sea-water in a 2-liter glass jar equipped with a stirrer to keep the solution in continuous motion. The toxicant was added through the aeration line. The air provided had been pumped through an in-line air filter; bubbled through 200-ml of o-DCB held in a 500-ml Erienmeyer flask; and pumped via aquarium air tubing to a capillary pipette (Figure 1).

The control solution was identical to the second solution except that the air was bubbled through distilled water rather than o-DCB (Figure 1).

All solutions were kept at 17°C throughout the experiment. The amount of air added to each solution was about 800-ml/minute. The concentrations of o-DCB in the control and vapor-added o-DCB solutions after 48 hours were 0.4 mg/liter and 21.1 mg/liter, respectively. A sample taken from the seawater portion of the liquid o-DCB/seawater solution after 48 hours of stirring and aeration had an o-DCB concentration of 75.8 mg/liter.

The test animals were purple sea urchins, Strongylocentrotus purpuratus. Adults were collected intertidally from Lunada Bay on the Palos Verdes Peninsula and acclimated for a week in the laboratory flow-through seawater system at 12°C. Males and females were then induced to spawn by injecting them with 1 to 2 ml of 0.5 M potassium chloride. The eggs were rinsed three times in seawater and then fertilized with sperm. (The procedure followed for spawning and fertilizing sea urchin eggs is well outlined in Hinegardner (1967).) The fertilized eggs, which divide and mature in near synchrony, were then added to the toxicant solutions, and aeration and stirring was initiated.

The measured densities of sea urchin eggs in the three solutions at 24 hours past fertilization were about 2.3 to 2.7 million eggs per liter. At regular intervals (0, 0.5, 1, 2, 3, 4, 5, 6, and 8 hours and every 2 hours until 52 hours), 2- to 4-ml samples of eggs and solution were pipetted into small vials and preserved with 2 percent borax-buffered formalin. (Only one 200- to 1,000-egg sample from each solution was examined at the end of each time interval.) The eggs were later examined with a com-pound microscope and their stage of development noted.

RESULTS

The eggs in the control solution displayed normal cell division and development throughout the 52 hours after fertilization: 98 percent of the eggs had reached the blastula stage in 5 hours and 94 percent were gastrulas in 28 hours (Figures 2 and 3). About 24 hours past fertilization, the first signs of invagination occurred with the formation of the primitive gut (the archenteron), and the sinking of the endodermal disc toward the animal pole (Figure 3). The influences exerted by the animal and vegetal poles appeared in fine balance, as normal development of the gastrula occurred. Development was normal in the gastrula to the pluteus stage; 89 percent of the embryos sampled at 52 hours were in the late prism or early pluteus stages.

Through 24 hours, the fertilized eggs that had been put in seawater with o-DCB vapors bubbled through showed development rates and patterns that were similar to those of the control embryos. However, past 24 hours, distinct differences between the two groups of embryos were apparent.

The embryos exposed to o-DCB vapors started to evaginate rather than invaginate their primitive guts. The endodermal disc did not move toward the animal pole but rather moved away from it forming an elongated process—the primitive gut "turned inside out" (Figure 3). At 52 hours, there were no normal gastrulas in this toxicant solution, and it was un-likely that any of the embryos would have achieved the pluteus stage. For purposes of further development and survival, all these embryos could be considered "dead."

The fertilized eggs that were added to the liquid o-DCB/ seawater solution did not undergo any further development. None of the eggs that were sampled showed evidence of even the first cleavage furrow. This solution immediately halted development.

DISCUSSION

Biesele (1958) has reported that some chlorinated benzenes are mitotic poisons that interfere with cell division and growth, and the abnormal sea urchin larvae produced by exposure to o-DCB in the present study might be a confirmation of the earlier findings. In a study in which brine shrimp (Artemia salina) were exposed to 10 ppm 1,3,5-trichloro-benzene for 24 hours, the overall reproductive performance of the adults was reduced by a factor of 10 (Grosch 1973). In the future, we hope to investigate the effects of low levels of o-DCB on the reproductive processes of adult sea urchins.

Ortho-dichlorobenzene is denser than seawater and thus tends to sink. This was easily observed in this study when the liquid o-DCB was poured into the seawater. In coastal water areas where municipal and industrial discharges occur, chlorobenzenes discharged may be accumulating on and within nearby sediments, thereby exposing benthic organisms to relatively high concentrations of these compounds. As de-scribed elsewhere in this annual report, there is evidence of some chlorobenzene accumulation in benthic fish collected near the Palos Verdes Peninsula. Chlorobenzene accumulation in animal and plant tissues may cause physiological difficulties for these organisms that could lead to a shortened life span. Alteration of normal reproductive or develop-mental patterns might also be expected, especially if dichlorobenzenes affect mitotic and meiotic mechanisms.

There were definite advantages in utilizing sea urchin embryos as test organisms since a great deal of biochemical, embryological, genetic, and toxicological work has previously been carried out on this type of organism. Partial life cycle toxicity tests that involve sensitive periods in the organism's life history, such as gastrulation, have been widely used. Difficulties specific to this preliminary experiment included not having enough o-DCB concentration measurements to adequately monitor o-DCB behavior in sea-water. Also, a special location for the 17°C refrigerator had to be found so that o-DCB vapors would not get into the laboratory and affect other animals.

Density measurements should be made continuously throughout the experiment to note the percentage of organ-isms that develop from egg to young pluteus. As it was, only those eggs and embryos that had not sunk or

decomposed were sampled for developmental observations. This procedure definitely biases the results toward higher survival, as the cells that had died were not accounted for.

This was a successful preliminary experiment. It was found that o-DCB can cause abnormal development at high sea-water concentrations. Future plans include tests involving dichlorobenzene concentrations that might be found in effluents and sediments and chemical studies to outline dichlorobenzene behavior in seawater.

Jack Mardesich and Theadore Heesen provided valuable assistance in setting up and monitoring this experiment. The guidance on handling the eggs and embryos provided by Dr. Susan Ernst, California Institute of Technology, Kerckhoff Marine Laboratory, was also appreciated.

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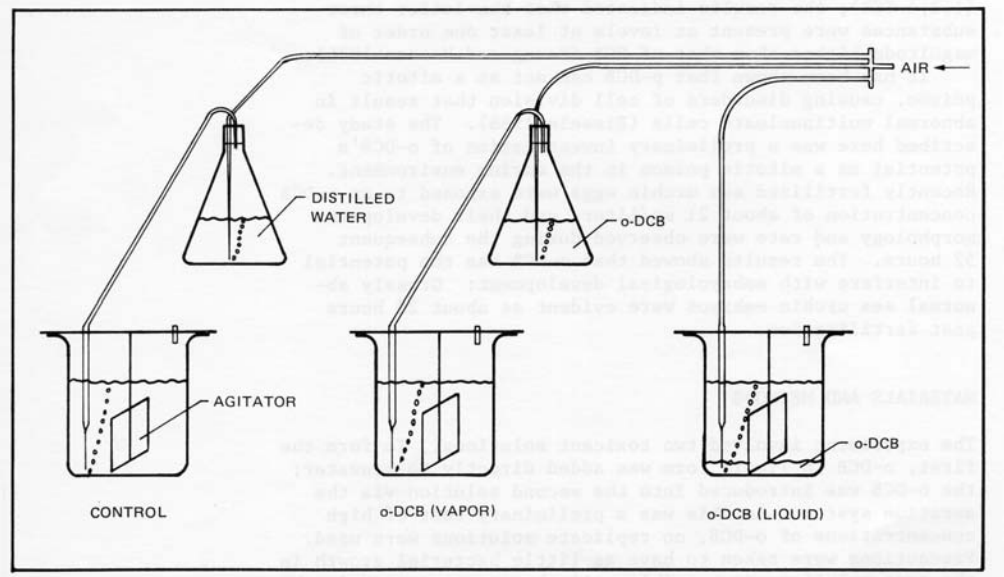

Figure 1. Configuration of equipment in test of toxicity of o-DCB to sea urchin embryos.

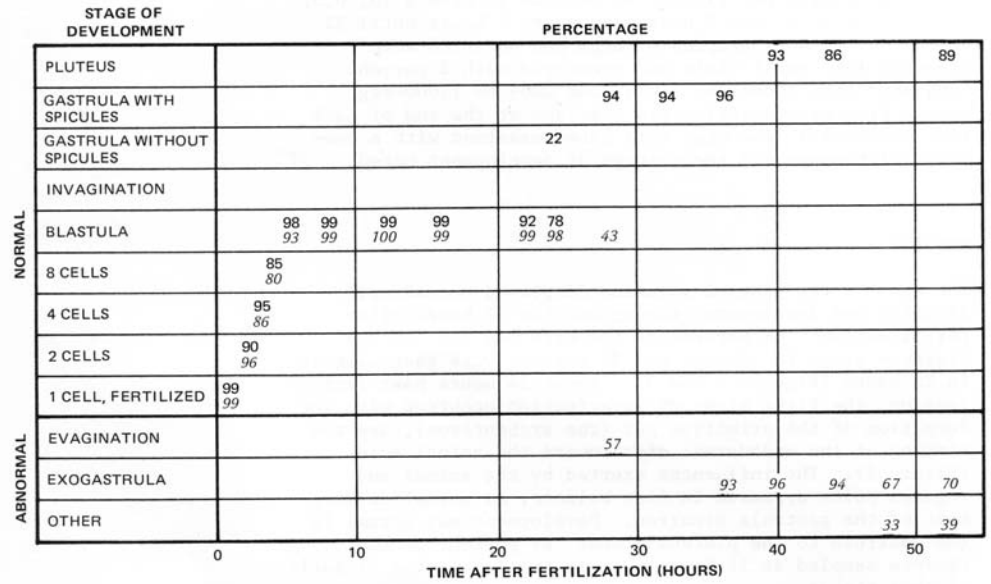

Figure 2. A summary of the development of sea urchin eggs in a test of o-DCB toxicity. Values are percentages of test organisms observed to have reached the stage listed in the left column at the time noted on the lower horizontal scale. Only major percentages (greater than 15 percent) have been shown. The upper number of each pair refers to the control organisms; the lower number (in italics) refers to those exposed to o-DCB.

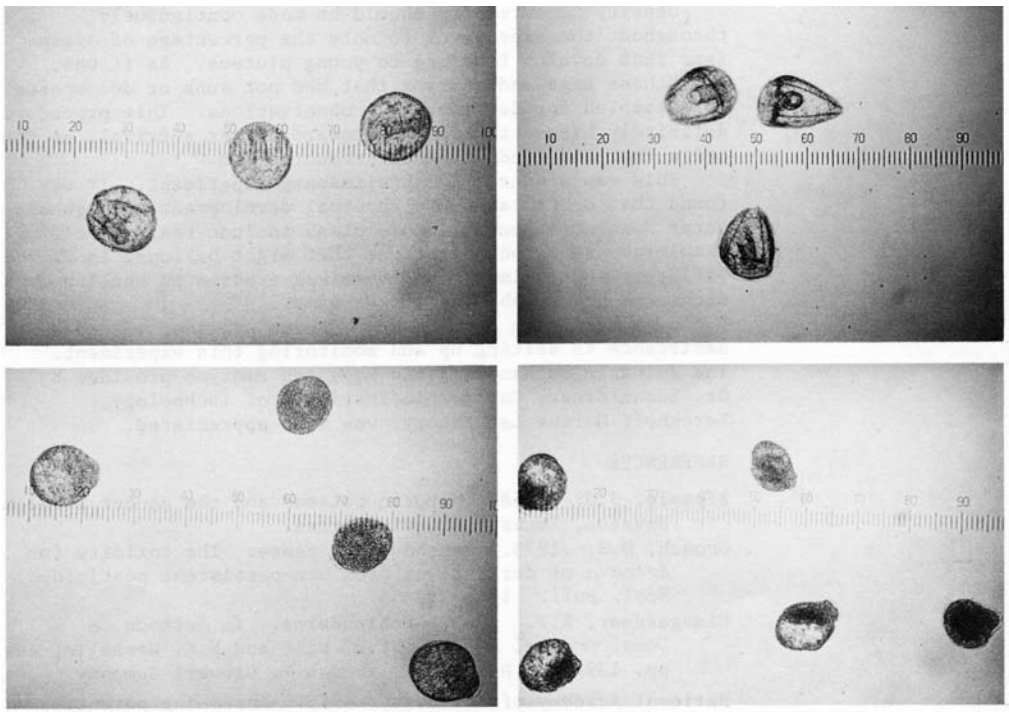

Figure 3. Development of sea urchin embryos in control and o-DCB solutions at 36- and 48-hours past fertilizations. Top left: 36-hour control. Top right: 48-hour control. Bottom left: 36-hour o-DCB. Bottom right: 48-hour o-DCB.