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Differential Response of Marine Organisms to Certain Metal and Agrichemical Pollutants

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It is common practice to evaluate trace pollutants in aquatic environments by chemical and physical analyses of the water, the sediments, or the content of the substances accumulated in certain biota (Lee and Mudd 1980). The chemical and physical approach is sound but it is laborious and expensive (Phillips 1977). Bioassays, which have other limitations, are comparatively less expensive and the time required to reach the endpoints is relatively short. This is particularly true when embryonic systems are used becuase embryonic period and certain developmental processes are short in time compared to the total life span of the organism (Dumont et al. 1982; Eaten et al. 1979; Lee and Xu 1983). Since embryonic forms are usually more sensitive than adults to adverse conditions, embryos and developmental events should be more superior as biological indicators for the assessment of pollutants in trace amounts. It was within this conceptual framework that the present investigation was undertaken.

Oocyte maturation of the starfish, fertilization and embryogenesis of sea urchins, and the development of amphioxus and brine shrimps were used to assay the effects of several common metals and agrichemicals frequently found in marine environments. While brine shrimp embryos were tolerant to metals and agrichemicals used here, sea urchins and amphioxus showed a differential response to the common metal pollutants. Starfish oocyte maturation process was affected by agrichemicals.

The results show that no one single organism, or its embryonic form, or a particular stage of development, can be used as the "indicator" for a particular pollutant. However, the use of lower forms of marine organisms can be useful collectively for environmental investigations and the management of waste disposal.

MATERIALS AND METHODS

All the test substances in artificial sea water (ASW) were prepared by a chemist and were supplied to the authors as coded samples. Decoding was done at the end of the experimental period. However, we did know which solutions contained metals or agrichemicals, but not the concentrations. The choice of organisms

tested with either agrichemicals or metals was based on availability. All experiments were carried out at room temperature, 20-25°C, in embryonic dishes in 4 ml of media. Observations were carried out with a dissecting microscope at 40X and also with phase optics at 100X. Two kinds of ASW were used. One was prepared from salt mixture purchased from Instant Ocean, Inc., Ohio, USA. Exact composition of this salt mixture, which contains trace elements, was not known because of "proprietory information." Other control ASW was prepared at the laboratory according to the MBL formula (Marine Biology Laboratory, Woods Hole, MA, USA) without the trace elements. 1-methyladenine was obtained from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals were reagent grade. The concentrations of the added metals represented the levels higher than the natural contents in uncontaminated sea water. All solutions were filtered through a membrane filter with 0.45 micrometer pores. The experiments were carried out from June to August, 1982, at the Institute of Oceanography in Qingdao, the People's Republic of China.

Fertilized eggs of the amphioxus, Branchiostoma belcheri tsingtaoense, were obtained from animals that were kept in aerated tanks with gravel bed as described by Tung et al. (1958). Each dish contained approximately 50 to 60 in 4 ml of solution. Since not all the eggs collected were fertilized eggs, only those that definitely showed clear fertilization membrane formation were considered as experimental animals.

Sea urchins, Temnopleurus toreumaticus, were kept in aquaria with running sea water. Spawning was induced by injection with 0.5 ml of 0.5M KCl. Eggs were washed several times with ASW before they were fertilized. Sperm were kept "dry" until use by diluting one drop to 10 ml with ASW. One drop of this diluted sperm suspension was used for fertilization. Fertilization and development were carried out in ASW or in test solutions, the concentrations of which are as indicated in the Results. To examine the effect of pollutants on morphogenesis, the eggs were fertilized in ASW and the fertilized eggs were then transferred to dishes containing test solutions.

Starfish, Patiria pectinefera, were kept in a running sea water tank. To obtain the oocytes, the ovary was dissected out and the eggs were liberated by gentle tearing of the ovary. The oocytes were then washed several times with ASW. To ascertain if the batch of oocytes collected at that particular time were fully grown and responsive to the meiosis inducing hormone, 1-methyladenine (1-MA), an aliquot of the washed oocytes was incubated in ASW containing 10^{-6}M 1-MA. If these oocytes responded to 1-MA, i.e., undergoing germinal vesicle breakdown (GVBD) within 60 min., this batch was used for experiments. To test the effect of the pollutants, the oocytes were incubated in the test solutions containing 10^{-6}M 1-MA.

Brine shrimp, Artemia salina (Tinjien strain), embryos were obtained as dessicated form from Tinjien Product Factory, the People's Republic of China, and they were incubated in all test solutions and control ASW.

RESULTS AND DISCUSSION

Brine shrimp embryos were most tolerant to all the substances at the levels tested. There were no effects on their hatchability or development.

The most toxic substance in effecting 1-MA induction of starfish oocyte maturation was triphenyls. Both the triphenyls showed a dose response effect while sodium pentachlorophenate (SP) showed the similar inhibitory effect at 50 ppm, 25 ppm, and 12.5 ppm on the meiosis induction activity of 1-MA (Table 1). At 0.75 ppm and 0.37 ppm, SP exerted no inhibitory effect (Table 2).

Table 1. Effects of Agrichemicals on 1-MA-induced GVBD in Starfish Oocytes

	% GVBD		
Concentration,* ppm	50	25	12.5
Triphenyl tin acetate	25%	53%	100%
Triphenyl tin fluoride	lyzed	54%	90%
Sodium pentachlorophenate	33%	34%	35%

*Stock solutions at 100 ppm

Table 2. Effect of Sodium Pentachlorophenate on 1-MA-induced GVBD of Starfish Oocyte

Concentration, ppm	Number of eggs examined	% GVBD
Control with no 1-MA	231	24.7
Control with 1-MA 10^{-6}M	250	100
50	337	23.6
25	290	21.3
12.5	196	21.4
6.25	243	32.9
3.17	267	48.7
1.5	266	39.1
0.75	372	100
0.37	428	100

Figures 1 and 2 represent the effect of several common metals on sea urchin fertilization and development on at least 80% of the viable embryos. The data is presented in this format to highlight the differential effects. Exact number was not determined because the embryos, including the abnormal ones, were free-swimming. The motility of the abnormal blastulae, gastrulae and plutei persisted as long as 4 days even though the development was blocked at the plutei stage (1-2 days post-fertilization).

Cu, Mg, Ni and Zn inhibited fertilization of sea urchin eggs as evident by the absence of fertilization membrane and cleavage (Fig. 1). Embryos at 0.5 ppm of Hg developed normally to blastulation but they developed abnormally afterward. All the plutei had abnormal morphology although they were still motile.

When sea urchin eggs were fertilized in control ASW and they were transferred to test solution, the fertilized eggs were able to develop except those in the Cu containing solutions (Fig 2). Hg and Zn at 0.5 and 10 ppm, respectively, exhibited a gastrulation block and the gastrulae were abnormal although the blastulae were normal. Embryos in Cd, Cr, Pb, Mg, Hg (0.1 ppm) and Ni developed normally to gastrulation stage. All the plutei were abnormal.

Four test solutions containing metals that affected the sea urchin the least were chosen to ascertain the usefulness of amphioxus as biological indicators. The solutions turned out to be Hg and Cd, each at two concentrations. Results (Table 3) show that Hg at 0.1 ppm and Cd at both 0.1 and 0.5 ppm had no effects on cleavage, i.e., 30-40 min. post-fertilization. At a higher concentration of Hg, 0.5 ppm, there was no development beyond the 8-cell stage. The neurulae, i.e., 14 hrs post-fertilization, were abnormal in Hg (0.1 ppm) and Cd solutions. The abnormality was diverse with mostly stunted and asymetric embryos. The results of the two Cd experiments at 14 hrs post-fertilization appeared to be contradictory. It is most likely due to the asynchronized nature of the development. The effects of Cd and Hg on amphioxus morphogenesis are evident.

The objective of this investigation is to utilize the sensitive nature of embryos of several marine organisms, whose developmental processes are known, to ascertain their suitability as biological indicator organisms for trace amounts of contaminants in marine environment. Because of their extensive usage in developmental and molecular biological studies, the handling of the organisms are now routine matter (Hinegardner and Tuzzi 1981). Allen (1977) and Vashchenko (1980) have taken similar approaches to study petrochemicals on the embryogenesis of sea urchins. Dumont and coworkers (1982, 1983), have used Xenopus embryos successfully for the study of water soluble coal extracts. The results of their investigations and recent studies from this laboratory (Lee and Xu 1982) definitively indicate that the approach taken and the use of certain organisms and their embryonic forms are useful and acceptable.

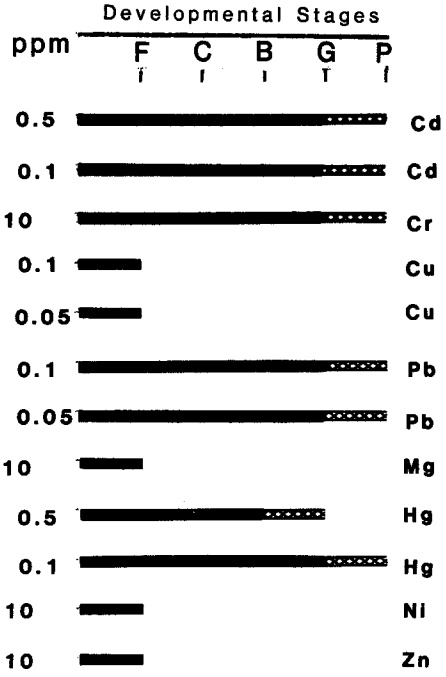

Figure 1.
Effect of Metals on Sea Urchin Fertilization and Development. Eggs were fertilized and allowed to develop in ASW containing metals. Ends of bars indicate development was up to that stage except for fertilization (see text for details). Solid bar represents normal development while hatched-bar represents abnormal development. F, fertilization; C, cleavage; B, blastulation; G, gastrulation; P, plutei.

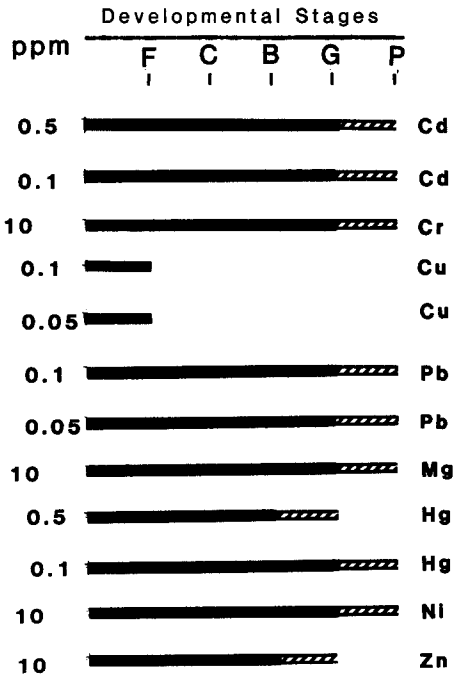

Figure 2.
Effect of Metals on Sea Urchin Development. Eggs were fertilized in control ASW and then transferred to test solutions. Other explanations as in Fig. 1.

Table 3. Effects of Hg and Cd on Amphioxus Development

Concentration	30-40 min post-fertilization		14 hrs post-fertilization	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Control ASW with trace element	8-16 cells	2-32 cells	normal neurulae	normal neurulae
ASW without trace element	4-16 cells	2-32 cells	normal neurulae	normal neurulae
Hg, 0.5	2 cells	4-8 cells	2 cells	4-8 cells
Hg, 0.1	4-8 cells	2-16 cells	all abnormal neurulae	all abnormal neurulae
Cd, 0.5	8-16 cells	2-32 cells	normal neurulae	16 normal 3 abnormal neurulae
Cd, 0.1	4-16 cells	2-32 cells	22 normal 3 abnormal neurulae	normal neurulae

Brine shrimp embryos would not be as good as other organisms as biological indicators because of their high degree of tolerance to the pollutants. Amphioxus embryos could be useful; however, their usefulness is limited because of the limited availability worldwide and their asynchronized nature in a population. Sea urchin embryogenesis and the maturation process of starfish oocytes are excellent candidates as biological indicator organisms.

Stage specific responses of one organism were revealed in this study. For example, fertilization of sea urchin eggs was inhibited by Cu, Mg, Ni, and Zn while cleavage was only inhibited by Cu and not by other metals. Amphioxus embryos, however, responded differently from the sea urchins to Hg and Cd. Cleavage of amphioxus was not affected by Hg and Cd while neurulation was. In another experiment using hormonal induction of oocyte maturation of starfish as an assay of agrichemical pollutants, one observed dose-response effects.

The findings of the differential response of different species and stage specific response to environmental pollutants can be utilized in a diversity of purposes. For example, in order to reach a short endpoint, the fertilization of sea urchin is more suitable

because successful fertilization only requires 15 to 30 minutes. To test the possible existence of teratogenic substances, one can use morphogenesis as markers, i.e., fertilization carried out in normal control solution while development and embryogenesis are carried out in test solutions. Using embryos rather than their adult counterparts, one will be able to distinguish non-specific toxicity and teratogenicity of certain substances. Starfish oocytes are good model systems not only for the investigation of hormonal effects on oocyte maturation but also for the other bioassay because of the existence of a simple but specific hormone, 1-methyladenine. Therefore, the effects of extrinsic agents on hormonal stimulation of oocyte maturation, as evident by GVBD, i.e., nuclear membrane dissolution and the resumption of meiosis, can be readily determined within 30-60 minutes. Since maturation of oocytes preempts embryogenesis, one then could predict fertilization and developmental effects which can then be used to predict future population density (Rosenberg et al. 1977).

Our studies here were with single pollutants at relatively low concentrations. In most cases, several pollutants are usually found together in one area. Interactions of several pollutants, whose concentrations may be below the physical-chemical detection, could elicit biological effects. The sensitivity of the assay system becomes the limiting factor, i.e., the more sensitive the better the system. Embryos of organisms such as Xenopus, sea urchins, and others whose embryology is known can, therefore, be most useful.

With respect to waste management, it is feasible to utilize the sensitivity and the breeding season of commercially important organisms as points of reference for disposal, both in time and in quantity. An oversimplified theoretical approach may be as follows. When the embryonic or larval phase of an organism is more sensitive to a certain pollutant than its adult form, that particular pollutant should not be released into the environment. Or, it should not be released at a harmful level. During the period when a particular organism of importance is more tolerant, higher quantities can theoretically be released. Since dilutions, accumulation and other physical-chemical-biological interactive processes participate in the recovery of an environment, all factors must be collectively considered.

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