Bull. Environm. Contam. Toxicol. 26, 207-212 (1981)

Effects of a Drilling Fluid on the Development of a Teleost and an Echinoderm*

Richard B. Crawford and Jonathan D. Gates

Department of Biology, Trinity College, Hartford, CT 06106, and Mt. Desert Island Biological Laboratory, Salsbury Cove, ME 04672

Offshore petroleum drilling operations introduce large quantities of drilling fluids (muds) into the marine environment. These drilling fluids are aqueous suspensions of various compositions pumped down the center of the drill bit. They provide the drilling process with lubrication, a suspending medium for the chips of material being drilled through, cooling, antibacterial action, protection against loss of oil or gas, and prevention of intrusion of water into the bore hole. Changes in the composition of the drilling fluid are made as new problems are encountered. During the drilling program, and particularly at its completion, portions or all of the drilling fluid may be discharged into the surrounding waters. This discharge can amount to significantly large quantities in the vicinity of a drilling platform.

The impact of drilling fluids on marine and estuarine environments is unknown due to the paucity of toxicological studies which have been conducted (TAGATZ et al. 1978). Among those aspects of living systems which need examining for sensitivity to these fluids are the earliest stages of development. We report here on the initial phases of studies on the effects of a drilling fluid sample on development of a teleost and an echinoderm embryo.

MATERIALS AND METHODS

Drilling fluid

The drilling fluid used was Control No. 16, 11, collected June 26, 1979 from a Mobile Bay drilling rig and supplied by the U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida 32561. The sample was a lignosulfonate-mud type containing barium sulfate. The stock material was kept at 4°. Solutions or suspensions prepared from the stock were kept at the temperature of the developing embryos. Standardization of the drilling fluid solutions was based on analyses

*Supported in part by N.S.F. Grant No. DEB-7826821 and N.I.H. Grant No. S07-RR-05764

0007-4861/81/0026-0207 $01.20

of dry weight. The dry weight of the stock was 262 mg/ml(262 ppt). Solutions to be tested were 10 ppt, 1 ppt, 100 ppm, 10 ppm, and 1 ppm, based upon dilution of the original drilling fluid with filtered seawater. The relatively stable suspension of drilling fluid developed a copious tan precipitate upon dilution with seawater. This precipitate interfered with observations of sand dollar development and therefore for these experiments the supernatant or seawater extract from dilution was used.

Fundulus development assay

Embryos of Fundulus heteroclitus (Linnaeus) were used as the model for teleost development studies. The adult fish were obtained from estuarine waters of Frenchman Bay, Maine, and kept in floating live cars in seawater. Gametes were obtained, fertilized, and embryos cultured according to descriptions found in NEW (1966) and CRAWFORD et al. (1973). Eggs were stripped from females and fertilization was initiated by adding a sperm suspension obtained by mincing dissected testes. Filtered sea water kept at 18° was the normal incubation medium. Developmental stage references used were those described by OPPENHEIMER (1973).

For these studies, embryos were placed in the drilling fluid solutions 1 min after fertilization and they were maintained at those concentrations for the duration of their development. The incubation dishes contained 10 ml of medium, changed daily, and from 50 to 100 embryos. The particularly susceptible events to environmental toxins observed with the dissecting microscope included early cleavage, blastulation, gastrulation, formation of the body axis, appearance of somites, yolk and body pigmentation, heart development and circulation, eye pigmentation, formation of organs and various fins, jaw and body movements, and hatching. At 18° the time from fertilization to hatching is approximately 35 days.

Sand dollar development assay

Embryos of the sand dollar Echinarachnius parma were used as the model for echinoderm development studies. Adults were collected in Frenchman Bay, Maine and were kept at 10° to minimize spontaneous spawning. Gametes were obtained by coelomic injection of about 1 ml of 0.5 M KCl, as described by HINEGARDNER (1967). Fertilization was begun by adding a few drops of a 1% sperm suspension to a suspension of eggs in about 100 ml of filtered seawater. The normal incubation medium was filtered seawater kept at 16°. The progress of development was observed with a compound microscope, particularly noting any effects on early cleavage, blastulation, hatching of the blastula to a spinning form, gastrulation, development of the prism and formation of the pluteus as described by KARNOFSKY and SIMMEL (1963). When noting the progress of pluteus development, attention was directed to motility and to the symmetry and lengths of the "arms". Approximately 10,000 embryos were placed in 30 ml of each incubation

medium 10 to 15 minutes after fertilization and they remained in these solutions for the duration of the experiment.

Assays for echinoderm egg fertilization

Gametes were pre-incubated for 15 min in the drilling fluid solutions prior to mixing. Fertilization was initiated by adding 5 drops of the 1% sperm suspension to 20 ml of egg suspension. Within two minutes the fertilized eggs may be identified microscopically as those with raised fertilization membranes. In each experiment, percent fertilization was determined by observing 100 eggs.

RESULTS

At early stages the drilling fluid appeared to have no effect on Fundulus development. By the seventh day marked effects could be observed at the higher concentrations. At 10 ppt and 1 ppt, the rate of development had been slowed, the embryos showed less pigmentation, and the cardiac and body movements were lethargic. Normal embryos at this time have well developed body axes and form, extensive pigmentation over the body and yolk, vigorous muscular twitches, and cardiac contractions and circulation.

The embryos in 10 ppt drilling fluid developed very slowly from the seventh day and by the 16th day they had become completely arrested. As seen in Table 1, the heart beat was severely affected in these embryos and by day 21 they were all dead. Embryos in 1 ppt drilling fluid were slowed in their developmental rate and heart beat. But by late stages they had caught up and were indistinguishable from the controls.

TABLE 1

Effect of Drilling Fluid on Cardiac Contractions in Fundulus Embryos

Drilling fluid in medium	Heart beats per minute[a]					
	14[b]	16	18	21	25	32
0(controls)	89	110	105	141	141	140
1 ppm	89	110	107	136	140	140
10 ppm	90	106	111	134	140	138
100 ppm	96	105	107	133	140	140
1 ppt	77	87	90	122	102	135
10 ppt	32	28	26	all dead -------		

[a] heart beats per minute determined by 30 second counts on at least 10 embryos.

[b] days post fertilization.

The hatching of embryos shown in Table 2 demonstrated another toxic effect from the drilling fluid. At 1 ppt the embryos hatched at about one-third the normal amount and at 100 ppm the hatching quantity was about one-half of that found in the controls. The fry which emerged appeared to be normal in all cases.

TABLE 2

Effect of Drilling Fluid on Hatching of Fundulus Embryos

Drilling fluid in medium	No of embryos[a]	No. hatched	% hatched
0	122	43	35
1 ppm	148	43	29
10 ppm	112	44	39
100 ppm	141	26	18
1 ppt	133	15	11
10 ppt	108	0	0

[a]Figures combined from two experiments

From these data it appears that Fundulus embryos can develop normally through hatching in concentrations of this drilling fluid sample up to 10 ppm. This represents a dilution of the original sample by approximately 26,000.

The sand dollar development is normal at concentrations of drilling fluid up to 100 ppm as seen in Table 3. At 1 ppt most of the embryos displayed delayed development followed by a variety of abnormal patterns. Only 30% of the embryos at this concentration formed normal plutei. At 10 ppt all embryo development was delayed and then arrested at the blastula stage.

TABLE 3

Effect of Drilling Fluid on Development of Sand Dollar Embryos

Drilling fluid in medium	Developmental Pattern[a]
0	Normal
1 ppm	Normal
10 ppm	Normal
100 ppm	Normal
1 ppt	Early development delayed. 30% normal plutei - remainder a variety of distorted forms.
10 ppt	Delayed from outset, arrested at blastula, remain alive and motile but no further development.

[a]To formation of very late pluteus.

Fertilization in sand dollars was affected by 15 min pre-incubation of gametes in drilling fluid as shown in Table 4. It is interesting to note that treatment of the sperm had no significant effect on fertilization, even at 10 ppt. When eggs were incubated in the drilling fluid at 10 ppt and 1 ppt, fertilization was virtually prevented.

TABLE 4

Effect of Drilling Fluid on Fertilization of Sand Dollar Eggs

Drilling fluid Treatment medium	Sperm-Egg Treatment combinations	% Fertilization
1 ppm	Normal sperm + Normal eggs	90
	Treated sperm + Normal eggs	90
	Normal sperm + Treated eggs	89
	Treated sperm + Treated eggs	90
10 ppm	Normal sperm + Normal eggs	90
	Treated sperm + Normal eggs	90
	Normal sperm + Treated eggs	90
	Treated sperm + Treated eggs	90
100 ppm	Normal sperm + Normal eggs	90
	Treated sperm + Normal eggs	83
	Normal sperm + Treated eggs	88
	Treated sperm + Treated eggs	88
1 ppt	Normal sperm + Normal eggs	90
	Treated sperm + Normal eggs	77
	Normal sperm + Treated eggs	6
	Treated sperm + treated eggs	4
10 ppt	Normal sperm + Normal eggs	93
	Treated sperm + Normal eggs	84
	Normal sperm + Treated eggs	1
	Treated sperm + Treated eggs	1

DISCUSSION

These data on effects of drilling fluid on embryo development in Fundulus and a sand dollar represent an initial survey on very general aspects of embryogenesis. It is clear that this drilling fluid sample contains toxic material, affecting the development of the teleost and the fertilization and development of the sand dollar.

It should be noted that no one sample is representative of all drilling fluids. The components of the fluid are altered to meet the momentary needs of the drilling operation. For example, this sample is moderately high in chromium (2400 µg/g), zinc (163 µg/g) and lead (66.6 µg/g) but low in barium content (7.31%)[1]. The range of variability among drilling fluids for these components can be found to be approximately 50 to 5500 µg/g for Cr, 50 to 600 µg/g for Zn, 25 to 120 µg/g for Pb, and 1 to 35% for Ba. This variability is not mentioned to suggest the active components, of which we have no knowledge, but only to illustrate the "non-typical" nature of any one sample.

In order that a proper assessment of the environmental impact of materials be made, the most sensitive stage of several potentially affected organisms must be determined. Therefore it is important to observe effects on fertilization, embryonic development, and early larval life. Furthermore, it will be necessary to test many different drilling fluids in order to obtain the range of toxicities one might expect from these substances. This will also assist in determining the specific toxic components present in these complex mixtures. From these initial studies, it is evident that further investigation of the toxicity of drilling fluids to the development of marine organisms is required.

REFERENCES

CRAWFORD, R.B., C.E. WILDE, M.H. HEINEMANN AND F.J. HENDLER: J. Embryol. Exp. Morph. 29, 363 (1973).

HINEGARDNER, R.T., Echinoderms, in Methods in Developmental Biology, Wilt, F.H. and Wessells, N.K., eds., New York: Thomas Y. Crowell Co. 1967.

KARNOFSKY, D.A. and SIMMEL, E.B.: Progr. Exp. Tumor Res. 3, 254 (1963).

NEW, D.A.T.: The Culture of Vertebrate Embryos. London: Academic Press 1966.

OPPENHEIMER, J.M.: Anat. Rec. 68, 1 (1937).

TAGATZ, M.E., IVEY, J.M., LEHMAN, H.K. and OGLESBY, J.L.: Northeast Gulf Science 2, 35 (1978).

[1]Metals analyses on drilling muds performed for EPA-Gulfbreeze by R.F. Shokes, Science Applications, Inc. and reported to N. Richards, April 4, 1980.