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Project Summary

Effects of Drilling Fluids on Embryo Development

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Toxicity of used drilling fluids to embryo development was investigated to ascertain the limits of safe usage of these fluids in marine environments. Embryos used as test systems were of the teleost, *Fundulus heteroclitus*, and four echinoderms *Echinarachnius parma*, *Strongylocentrotus purpuratus*, *Lytechinus pictus*, and *Lytechinus variegatus*. Gametes were collected in season and combined for fertilization; the resultant embryos were reared in various concentrations of the drilling fluids. Any departure from normal developmental patterns was noted and the embryotoxicity for each fluid determined. The drilling fluids tested came from various sources; 24 different samples were evaluated. In addition, several commercial drilling fluid components were examined in the test systems including a synthetic reference mud.

These studies demonstrated that no single drilling fluid is "typical" and that the quantitative effects on embryos vary considerably from one fluid to another. Some drilling fluids are quite toxic to one or more of the embryo systems, requiring dilutions of over 10^5 to become "safe." Others are relatively innocuous, requiring very little dilution to achieve a concentration in which embryo development can proceed normally. Also, the effects on development show variation from one fluid to another, thus indicating that a variety of components or compounds are responsible for toxic manifestations. Identification of the components responsible for toxicity must await chemical analysis of these fluids.

This Project Summary was developed by EPA's Environmental Research Laboratory, Gulf Breeze, FL, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

As a result of offshore petroleum drilling, large quantities of drilling fluids (muds) are introduced into the marine environment. These drilling fluids are aqueous suspensions of a variety of components pumped down the center of the drill bit. The composition of such fluids varies with the needs of the drilling operation, such as lubrication, cooling, prevention of intrusion of seawater into the bore hole, antibacterial action, suspension of drill cuttings, and capture of H_2S. The drilling fluid may be partially or wholly discharged into the surrounding waters during drilling, especially at its completion. 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 has been largely unknown due to the paucity of toxicological studies. Recently, a few studies on biological parameters affected by drilling fluids have been reported. Effects on the chemosensory neurons in the walking legs of the lobster have been examined. The impact of drilling fluids on the distribution of organisms in benthic communities has been measured. Several marine invertebrates, as well as some teleosts, have been tested for their survival in dilutions of drilling fluids. In each case, some drilling fluids at appropriate concentrations have been shown to have adverse effects on the biological model.

The studies reported here were carried out to examine in some detail the effect of several representative drilling fluids on fertilization and embryo development. For model systems, embryos of an estuarine teleost, *Fundulus heteroclitus*, were chosen

as well as embryos of four echinoderms, *Echinarachnius parma, Strongylocentrotus purpuratus, Lytechinus pictus,* and *Lytechinus variegatus.* These models represent a variety of habitats and embryo types which would be found in the vicinity of petroleum drilling operations. In addition, each of these species represents an important member of food chains. Conditions which might interfere with the reproductive capacity of the species would have effects more far reaching than simply on the species alone.

Design of Experiments

Models for the study of effects on embryogenesis must be easily obtained and easily reared in the laboratory. Also, they should represent species found in the locale of interest. Furthermore, it is helpful if the species has been extensively studied so that conditions for its use may be anticipated. All these criteria were met by the species used in these studies.

Because *Fundulus* eggs and sperm were obtained in season, the time of fertilization could be controlled. Within one minute of fertilization, the zygotes were placed in various seawater dilutions of the drilling fluids. The embryos developed in those fluids until a few days past hatching (35 days) and the various aspects of development were observed daily. Any departure from observed normal development was considered a measure of the toxicity of the drilling fluid.

Sand dollar and sea urchin gametes were obtained in season by intracoelomic injection of KCl. Within 15 minutes after fertilization, the embryos were placed in the drilling fluid solutions and development was observed. Rearing these embryos through the pluteus stage takes about four days and therefore an assay may be accomplished quite quickly. In addition, the gametes may be pre-incubated in drilling fluid solutions to test the effects of these substances on fertilization.

Drilling fluids of various types and from scattered regions were obtained from the Environmental Research Laboratory of the United States Environmental Protection Agency, Gulf Breeze, Florida. These fluids were stored at 4°C and, when dilutions were made for testing, were kept at the temperature used for rearing the embryos. Commercial components of drilling fluids were also obtained from the U.S. EPA and were tested for their toxicity.

Drilling fluid test solutions were standardized according to dry weight. Since the dry weights of the fluids showed great variation (from 1300 mg/ml to 135 mg/ml) different amounts of dilution were required to achieve the standard stock suspension of 10 parts per thousand (ppt). This suspension was stirred for 1 hour and then filtered through Whatman #1 paper. Further dilutions were made by diluting the 10 ppt stock with filtered seawater.

Results

Fundulus development was not affected by any of the drilling fluids during early stages. At the onset of organogenesis, some fluids caused a diminution of heart beat rate at 10 ppt. The most significant effect was on hatching and on coordination of swimming of the fry. Adverse effects on these activities could be achieved at concentrations of 1 ppt with some fluids.

Echinoderm egg fertilization was prevented by most drilling fluids at concentrations of 10 ppt. Many of the fluids also significantly reduced the fertilization rate at 1 ppt. Some of the components were effective against fertilization at 10 parts per million (ppm), especially those containing lignosulfonates or detergents.

Development of the echinoderm embryo was the most sensitive test with these drilling fluids; adverse effects were observed in some cases at 100 ppm and 10 ppm. Developmental arrest or distortions occurred at a variety of stages, depending on the fluid tested. Also, the commercial drilling fluid components, especially those containing lignosulfonates or detergents, inhibited development at low concentrations.

From the data it is clear that drilling fluids vary to a considerable extent regarding their toxicity. By calculating actual dilutions of the fluids required to achieve toxic manifestations, "safe" dilutions, or concentrations of the drilling fluids which yield no adverse effects in these assay systems, can be determined. Such "safe" dilutions range from 135 to 1,132,000 (least toxic to most toxic). That is, one of the fluids must be diluted about one-million-fold to allow normal development of one of the sea urchins. On the other hand, another fluid is "safe" by diluting it slightly more than one-hundred-fold.

Conclusions

Clearly, drilling fluids contain toxic materials, substances which interfere with fertilization and normal development. It is also clear that no one fluid is typical. Since the range of toxicity varies greatly from one fluid to another, it is not always possible to prejudge the effect on marine life of a large discharge of drilling fluids from a drilling rig; each fluid must be tested, or an accurate log kept of the makeup of the fluid compared with log data from previously tested fluids. Even with such logs, the effects of cuttings from the bore hole on the toxicity of the fluid would not be known.

Further, accurate determinations of the actual dilutions of discharges from rigs must be known. If a dilution of one million fold were easily obtained a short distance from a rig, it would seem that the most toxic drilling fluid is harmless to the animal systems tested in these studies. Also, more data are needed about chemical or physical changes in the drilling fluid that might render it more or less toxic in seawater over a given period of time.

Comparison of chemical analyses of these drilling fluids with their toxicities may yield interesting and useful conclusions regarding those components which are harmful.

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***Thomas W. Duke** is the EPA Project Officer (see below).*
The complete report, entitled "Effects of Drilling Fluids on Embryo Development," (Order No. PB 83-190 983; Cost: $8.50, subject to change) will be available only from:

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