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TOXICITY OF DRILLING-MUD BIOCIDES TO DEVELOPING ESTUARINE MACROBENTHIC COMMUNITIES¹

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ABSTRACT: The effects of various biocides, as used in drilling muds for exploratory drilling for oil offshore, were determined by comparing macrobenthic communities that developed from planktonic larvae in treated and untreated aquaria. Surflo®-B33 and Aldacide® were tested simultaneously. Harvest at seven weeks yielded 1,941 animals, representing 37 species of 6 phyla. The effects of Surflo-B33 (25% dichlorophenol and other chlorophenols) on these communities were similar to those of pentachlorophenol (Tagatz *et al.*, 1977; 1978), a biocide known to be toxic to many aquatic organisms (Rao, 1978). Significantly fewer ($\alpha = 0.05$) chordates, mollusks, and annelids occurred in 819 μg Surflo-B33/l (measured) than in the control; mollusks were most sensitive and also significantly fewer in aquaria receiving 41 $\mu\text{g}/\text{l}$. Average numbers of animals and species per aquarium did not significantly decrease in Aldacide (91% paraformaldehyde) at nominal concentrations of 15 and 300 $\mu\text{g}/\text{l}$. The high toxicity of the chlorophenols tested indicates that use of alternative biocides, possibly paraformaldehyde, should be considered for natural waters.

Biocides (bactericides) are added to drilling muds and completion fluids used in offshore oil exploration to suppress microbial degradation of organic additives and the formation of hydrogen sulfide by sulfate-reducing bacteria. Types of biocides currently used include aldehydes, chlorinated phenols, quaternary amines, diamine salts, and other chemicals, such as caustic, alkyl phosphates and heavy metal salts (Bureau of Land Management, 1978). Their efficacy, treating rates, and environmental effects must be substantiated for registration with the Environmental Protection Agency (EPA) (Robichaux, 1975).

Although most of the drilling mud is recovered and recycled, tons of mud and drill cuttings are discharged and deposited on the bottom near drilling

sites (Shinn, 1975; George, 1975). Of the mud constituents, biocides reportedly are particularly toxic to aquatic biota and deserving of special consideration (Bureau of Land Management, 1978). A Bureau of Land Management (1978) impact statement cautions that the concentrations of pentachlorophenol recommended for use, 0.25 to 0.50 lbs (113 to 227 g) per barrel, and current drilling fluid disposal practice could introduce large quantities of this compound into the marine ecosystem. The toxicity of pentachlorophenol to a wide variety of aquatic organisms is well documented (Rao, 1978), however, the relative toxicity of other biocides used in drilling operations is little known.

We initiated studies to determine the effects of the biocides, Aldacide® (active component, paraformaldehyde) and Surflo®-B33 (active component, sodium

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salts of 2,2'-methylenebis and other chlorophenols), on the development of estuarine macrobenthic communities.² Earlier similar experiments conducted with pentachlorophenol (PCP) used technical PCP and PCP product, Dowicide®G-ST that contained 79% sodium pentachlorophenate, 11% sodium salts of other chlorophenols, and 10% inert ingredients (Tagatz *et al.*, 1977; 1978).

Studies reviewed by Land (1974) indicated that the acute toxicity of formaldehyde to six species of marine and freshwater fishes ranged from 25 to 60 parts per million (ppm); toxicity to freshwater daphnids (*Daphnia* sp.) was 2 ppm. He stated that the high sensitivity of fishes to Dowicide G indicates that formaldehyde is preferential for drilling fluids, but the higher toxicity of formaldehyde to invertebrates should be considered.

This study and our comparable earlier studies on pentachlorophenol provide data on the relative effects of three biocides on developing epibenthos in experimental communities.

METHODS AND MATERIALS

The effects of Aldacide and Surflo-B33 were determined by comparing numbers and species of animals that grew from planktonic larvae in treated aquaria for seven weeks (July 31 to September 18, 1978) with animals that grew in untreated aquaria. Larvae entered the aquaria from the natural component of plankton in flowing unfiltered seawater. We used five plexiglass apparatuses, each consisting of a central constant-head box from which eight aquaria (40

cm long, 10 cm wide, 12 cm high) extended (Fig. 1). Aquaria were filled to a depth of 5 cm with clean silica sand (particle sizes: 75%, 354 to 500 μ m; 96%, 250 to 710 μ m) dredged more than a year previously from Santa Rosa Sound, Florida. Water depth in aquaria was maintained at 8 cm (3 cm above substratum).

Seawater with its constituent plankton was pumped from Santa Rosa Sound to a splitter box where five adjacent glass tubes supplied water at a rate of 2 l/min to the five constant-head boxes that supplied water to the aquaria. Flow to each aquarium was maintained at 200 ml/min by adjusting the height of a 3-mm diameter hole in the wall of the constant-head box. Water flowed from each aquarium through a notched end-opening. Salinity of the water averaged 25 ‰ (18 to 30 ‰); temperature, 29°C (26 to 30°C).

Each biocide dissolved in deionized water was metered by pump into and mixed with the seawater entering the center of the constant-head box of each treated apparatus. One apparatus of eight aquaria received no toxicant and served as the control.

The commercial form of each biocide was tested. Manufacturer labels indicate that Aldacide consists of 91% paraformaldehyde (polymerized formaldehyde (CH₂O)_n) and 9% inert ingredients. Surflo-B33 consists of 37.4% isopropyl alcohol, 17.0% sodium salt of 2,2'-methylenebis (4,6-dichlorophenol), 8.0% sodium salts of other chlorophenols (identity undetermined), and 37.6% inert ingredients.

Concentrations of Surflo-B33 in seawater per apparatus of eight aquaria were measured (nominal, 40 and 800 μ g/l, parts per billion); concentrations of Aldacide were nominal, 15 and 300 μ g/l (we had no method for measuring con-

² ©Aldacide and Surflo-B33 (Baroid Petroleum Service/NL Industries, Inc., Houston, Texas). Dowicide G-ST (Dow Chemical Co., Midland, Michigan). Mention of commercial products does not constitute endorsement by the EPA.

Figure 1. Apparatus used to test the effects of Aldicide and Surflo-B33 on development of estuarine macrobenthic communities. The splitter box supplied seawater to five apparatuses (not to four as shown), and a reciprocating pump delivered toxicant (not a syringe pump as shown). From Tagatz *et al.*, 1979.

centrations of Aldicide at the time of this study). Concentrations were similar to those used in our earlier PCP experiments (based on active ingredient, a.i., of each compound). A relatively high concentration (a.i.) of Aldicide was tested because manufacturer recommendations for Aldicide are about 300 g/bbl (42 gal., 16 dkl.) of drilling fluid and for PCP and Surflo-B33, about 150 g/bbl.

Samples of water were taken from the constant-head boxes of the two apparatuses treated with Surflo-B33 twice a week for analysis for toxicant concentration. Determinations of Surflo-B33 concentrations were made on a Hewlett-Packard Model 5710A gas-liquid chromatograph. Operating conditions were: ^{63}Ni electron capture detector; detector, 300°C ; injector, 150°C ; column, 0.9 m X 2 mm I.D. 1% SP 1240 DA; column, 135°C ; and carrier gas, 10% argon/methane at 25 ml/min. Analysis of a sample of our Surflo-B33 stock provided the standard for calculations of Surflo-

B33 in our water samples. Our one-liter water samples were acidified to pH = 2 and extracted with two 100-ml portions of 1:1 diethyl ether/petroleum ether (v/v). The extracts were filtered through glass wool into a 500 ml Kuderna Danish concentrator and evaporated on a steam table to 25 ml and further evaporated under a gentle stream of nitrogen to the appropriate volume for chromatograph analyses. Limit of detection is 1.0 ppb. Average recovery from fortified seawater exceeded 85%; data were not adjusted for recovery.

After seven weeks, animals were collected by siphoning contents of the aquaria into a 1 mm-mesh sieve, preserved, and later identified. For each biocide, one-way analysis of variance and Duncan's *post hoc* analysis of concentration means were used to compare numbers of animals in control and treated aquaria ($\alpha = 0.05$). Results are presented as pooled data for each toxicant concentration and the control.

RESULTS

The averages and ranges of measured concentrations of Surflo-B33 in water from the two apparatuses treated with this toxicant were 41 $\mu\text{g/l}$ (30 to 67 $\mu\text{g/l}$) and 819 $\mu\text{g/l}$ (578 to 1215 $\mu\text{g/l}$). Concentrations of Aldicide were nominal (15 and 300 $\mu\text{g/l}$); daily monitoring of the volume of toxicant solutions indicated correct amounts were introduced into the aquaria.

Our harvest at seven weeks yielded 1,941 animals representing 37 species of 6 phyla (Table 1). Numerically dominant phyla were Chordata, Mollusca, and Annelida.

Abundance of animals was decreased significantly ($\alpha=0.05$) by Surflo-B33, but not by Aldicide (Table 2). Average numbers of individuals and species per aquarium were significantly less in aquaria receiving 819 $\mu\text{g/l}$ Surflo-B33 than in control aquaria or those receiving 41 $\mu\text{g/l}$. Significantly fewer chordates, mollusks, and annelids occurred in 819 $\mu\text{g/l}$. Mollusks were most sensitive and abundance was significantly reduced in 41 $\mu\text{g/l}$. In addition, no coelenterates, *Aiptasis pallida*, occurred in the high concentration although 9 were present in the control and 6 in the low concentration.

Sensitivity to Surflo-B33 differed among species of the same phylum, but characteristically not among species of the same class or family. Thus, for the two most abundant mollusks collected, *Mulinia lateralis* and *Acteocina canalliculata*, the abundance of the former (a pelecypod) but not of the latter (a gastropod) was affected by 41 $\mu\text{g/l}$. Species difference was not evident in 819 $\mu\text{g/l}$ because, except for a single individual, both species were absent. Of the three most abundant annelids collected, *Polydora ligni* (Spionidae) was fewer in 41 and 819 $\mu\text{g/l}$ than in the

control, *Mediomastus californiensis* (Capitellidae) was fewer only in 819 $\mu\text{g/l}$, and *Neanthes succinea* (Nereidae) was more abundant in both concentrations than in the control.

Average numbers of animals and species per aquarium did not significantly decrease in the presence of Aldicide. The number of chordates in 15 $\mu\text{g/l}$ was significantly higher than the number in the control. We do not know why environmental conditions were particularly favorable for chordates in all 15 $\mu\text{g/l}$ aquaria, but this increase is probably spurious because abundance in 300 $\mu\text{g/l}$ was unaffected.

DISCUSSION

The effects of Dowicide G-ST (PCP and other chlorophenols), Surflo B-33 (dichlorophenol and other chlorophenols), and Aldicide (paraformaldehyde) on the major phyla of macrobenthic animals collected in community studies are compared in Table 3. Comparison of relative toxicity is based on concentrations of the active ingredients of the compounds tested, although other components such as solvents and inert ingredients possibly could add to their toxicity.

PCP (alone or with other chlorophenols) or dichlorophenol (with other chlorophenols) significantly reduced the numbers of individuals of annelids, arthropods, chordates, and mollusks in the benthic communities; paraformaldehyde did not significantly reduce numbers at the concentrations tested. Mollusks were particularly sensitive to all concentrations of PCP and dichlorophenol. Paraformaldehyde had no significant adverse effects on numbers of annelids, chordates, and mollusks in the experimental communities; too few arthropods were collected to indicate any effects on this phylum. When all phyla collected in each test are con-

Table 1. - Animals collected from control aquaria and from aquaria exposed to Aldacide or Surflo-B33 for seven weeks. Replicates were pooled.

Animal	Control	Aldacide μg/l		Surflo-B33 μg/l	
		15	300	41	819
CHORDATA					
<i>Molgula manhattensis</i>	179	370	190	234	2
MOLLUSCA					
<i>Mulinia lateralis</i>	139	84	108	57	0
<i>Acteocina canaliculata</i>	42	59	38	43	1
<i>Lyonsia hyalina floridana</i>	9	10	14	8	1
<i>Diastoma varium</i>	8	6	5	0	0
<i>Amygdalum papyrium</i>	0	7	6	2	1
<i>Musculus lateralis</i>	1	0	3	6	0
<i>Laevicardium mortoni</i>	1	1	2	1	0
<i>Anadara transversa</i>	1	3	0	0	0
<i>Crassostrea virginica</i>	0	1	1	0	0
<i>Cyrtopleura costata</i>	1	0	1	0	0
<i>Dinocardium robustum</i>	0	1	1	0	0
<i>Abra aequalis</i>	0	0	1	0	0
<i>Mitrella lunata</i>	1	0	0	0	0
Total	203	172	180	117	3
ANNELIDA					
<i>Mediomastus californiensis</i>	14	3	7	18	4
<i>Neanthes succinea</i>	3	11	7	12	9
<i>Polydora ligni</i>	18	2	6	5	0
<i>Eupomatus dianthus</i>	14	6	2	0	4
<i>Capitomastus aciculatus</i>	8	11	4	0	0
<i>Nereis pelagica</i>	1	5	8	5	0
<i>Podarke near guanica</i>	1	2	3	3	2
<i>Capitella capitata</i>	4	3	2	1	0
<i>Cistenides gouldii</i>	2	4	1	1	0
<i>Pherusa affinis</i>	2	1	2	2	0
<i>Armandia maculata</i>	0	0	1	2	0
<i>Marphysa sanguinea</i>	0	2	1	0	0
<i>Diopatra cuprea</i>	0	1	0	1	0
<i>Dasybranchus lunulatus</i>	0	0	1	1	0
<i>Pomatoceros</i> sp.	0	0	2	0	0
<i>Capitellides jonesi</i>	0	1	0	0	0
<i>Prionospio heterobranchis texana</i>	0	0	0	1	0
Total	67	52	47	52	19
COELENTERATA					
<i>Aiptasia pallida</i>	9	11	12	6	0
ARTHROPODA					
<i>Balanus amphitrite niveus</i>	3	1	1	3	0
<i>Corophium acherusicum</i>	0	0	1	2	1
<i>Balanus eburneus</i>	0	0	0	1	0
<i>Rhithropanopeus harrisii</i>	0	0	0	1	0
Total	3	1	2	7	1
ECHINODERMATA					
<i>Leptosynapta inhaerens</i>	0	0	0	2	0
ALL PHYLA					
Individuals	461	606	431	418	25
Species	22	25	29	25	9

Table 2. - Average density of animals and number of species per aquarium, by phylum (range in parentheses), collected from control aquaria and aquaria exposed to Aldicide or Surflo-B33 for seven weeks.

Phylum	Control		Aldicide ($\mu\text{g/l}$)				Surflo-B33 ($\mu\text{g/l}$)			
	No.	Sp.	No.	Sp.	No.	Sp.	No.	Sp.	No.	Sp.
Chordata	22.4 (7-55)	1 (1)	46.2 (28-66)	1 (1)	23.8 (14-37)	1 (1)	29.2 (9-45)	1 (1)	0.2* (0-2)	0.1* (0.1)
Mollusca	25.4 (15-34)	3.8 (2-6)	21.5 (12-28)	4.4 (3-6)	22.5 (14-29)	4.8 (3-6)	14.6* (9-23)	3.1 (2-5)	0.4* (0-2)	0.4* (0-2)
Annelida	8.4 (4-13)	4.5 (2-7)	6.5 (2-12)	4.0 (2-6)	5.9 (4-8)	4.2 (3-6)	6.5 (4-11)	4.1 (2-8)	2.4* (0-4)	1.8* (0-4)
Coelenterata	1.1 (0-3)	0.5 (0-1)	1.4 (0-3)	0.8 (0-1)	1.5 (0-5)	0.6 (0-1)	0.8 (0-2)	0.5 (0-1)	0 (0)	0 (0)
Arthropoda	0.4 (0-1)	0.4 (0-1)	0.1 (0-1)	0.1 (0-1)	0.2 (0-1)	0.2 (0-1)	0.9 (0-3)	0.9 (0-3)	0.1 (0-1)	0.1 (0-1)
Echinodermata	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0-2)	0.1 (0-1)	0 (0)	0 (0)
All phyla	57.6 (33-99)	10.0 (8-13)	75.8 (47-95)	10.2 (8-15)	53.9 (41-64)	10.9 (9-13)	52.2 (39-65)	9.8 (6-15)	3.1* (0-6)	2.4* (0-4)

*Significantly less than control at 5% level; significance is indicated only for the three most abundant phyla.

sidered, all but the lowest concentrations of the chlorophenols significantly affected the total number of individuals colonizing the communities; again, paraformaldehyde had no significant adverse effect.

These combined studies indicate that chlorophenol-type biocides, at the concentrations tested, adversely affect benthic colonization by planktonic larvae. Reduction in the density of macrofauna may be due to direct effects of biocides on colonization or survival and to secondary effects, not assessed in this study, of changes in bacterial or meiofaunal populations. Any decrease in macrofauna in nature could lead to a local reduction of recruitment that could impact other organisms in aquatic food webs.

These studies also indicate that the effects of Surflo-B33 (dichlorophenol and other chlorophenols) to experimental settling communities were

similar to those of PCP, known to be toxic to many aquatic organisms; whereas Aldicide (paraformaldehyde) had no significant adverse effect on the number of macrofauna colonizing these communities. Alternative biocides, possibly paraformaldehyde, should be considered for use in the natural environment. However, toxicity experiments provide only part of the information needed before relative environmental hazards can be assessed. Data on potential for bioaccumulation and fate data necessary for prediction of environmental concentration are also critically important.

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Table 3. - Relative effects of pentachlorophenol (PCP), dichlorophenol, and paraformaldehyde type biocides on the number of animals in macrobenthic communities. Concentrations of the chlorophenols are based on measured concentrations; paraformaldehyde, on nominal concentrations. Data on PCP from Tagatz *et al.*, 1977; 1978.

Phylum	PCP		PCP		Dichlorophenol and other chlorophenols		Paraformaldehyde	
	Technical grade		and other chlorophenols		and other chlorophenols			
	$\mu\text{g/l}$	$\mu\text{g/l}$	$\mu\text{g/l}$	$\mu\text{g/l}$	$\mu\text{g/l}$	$\mu\text{g/l}$	$\mu\text{g/l}$	$\mu\text{g/l}$
	7	76	18.0	183	10.2	205	13.6	273
Mollusca	*	*	*	*	*	*	NS	NS
Annelida	NS	*	NS	*	NS	*	NS	NS
Arthropoda	NS	NS	NS	*	—	—	—	—
Chordata	—	—	—	—	NS	*	NS	NS
All phyla	NS	*	*	*	NS	*	NS	NS

* = Number of individuals significantly less than control ($\alpha = 0.05$).
NS = Not significantly less than control.
— = Relatively few collected.

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