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2,2-Dibromo-3-Nitrilopropionamide, a Compound with Slimicidal Activity

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Laboratory and field tests demonstrated that 2,2-dibromo-3-nitrilopropionamide was an effective slimicide for use in papermaking systems and cooling towers. It was also effective as a bactericide for soluble oil emulsions. Acute toxicity tests showed that its hydrolysis at *p*H 9 and 23 C yielded products that were relatively nonhazardous to fathead minnows.

The purpose of this paper is to present new information about the use of 2,2-dibromo-3-nitrilopropionamide (DBNPA) as an industrial slimicide. Included are its chemical, physical, toxicological, and antimicrobial properties, as well as efficacy under conditions of use.

In 1947, Nolan and Hechenbleikner (U.S. patent no. 2,419,888) cited the use of bromoacetamides to protect seeds and seedlings from soil-borne organisms which cause seed rot, seedling blight, and damping off. Their patent mentions the fungal diseases caused by *Pythium*, *Rhizoctonia*, and *Fusarium*.

MATERIALS AND METHODS

DBNPA was prepared by the method described by Hesse (3). The white product which resulted from bromination in an aqueous medium was recrystallized from benzene to give a compound with a melting point of 125 C. Purity was checked by elemental analysis, infrared analysis [IR (Nujol mull), 1,710 cm^{-1} (C=O)], and by nuclear magnetic resonance spectroscopy [NMR (dimethyl sulfoxide-d_6), 8.36 δ (doublet)].

Physical properties. Table 1 summarizes the solubility of DBNPA in solvents commonly used. Although this compound is relatively soluble in water, its rate of solution can be increased by wetting it with acetone or *N*,*N*-dimethyl formamide before addition to water.

Chemical properties. The white, crystalline DBNPA has been stable for at least 4 years under laboratory storage conditions. This conclusion is based upon no detectable change in appearance or biological activity during this storage period.

DBNPA dissolves in water to give a relatively stable solution in an acid *p*H range. Its unusual solubility and stability in polyethylene glycol (average molecular weight, 200) make this glycol a preferred solvent.

Aqueous solutions hydrolyze under alkaline conditions, with the rate of decomposition increasing with the alkalinity. However, the rate of hydrolysis is not fast enough to interfere with the antimicrobial activity of fresh, alkaline (*p*H 7 to 9.5) solutions.

Heat and ultraviolet and fluorescent light also cause aqueous solutions of DBNPA to degrade, as evidenced by the change of the antimicrobial end point as a given solution ages. This decomposition has also been substantiated by chemical analysis.

Toxicological properties. Animal tests, carried out in our toxicological laboratory, have indicated that DBNPA is moderately toxic. The 50% lethal dose (LD_{50}) value ranges from 118 mg/kg of body weight for female guinea pigs to 235 mg/kg for male Sherman rats. Eye-contact tests on laboratory animals indicate that DBNPA damaged the eye seriously enough to cause possible impairment of vision. A single, short skin exposure to DBNPA should result in no significant irritation. A single prolonged or frequently repeated skin exposure, however, may result in irritation, even a burn, depending on the severity of the exposure. Based on animal tests, this material is not likely to be absorbed through the skin in acutely toxic amounts (5).

The acute fish toxicity of fresh and aged solutions of DBNPA has been determined for fathead minnows, *Pimephales promelas* Rafinesque, using dechlorinated Lake Huron water at 50 F and 72 hr of exposure (2). A fresh solution killed the minnows above 1 μg/ml, but parallel studies of solutions aged at *p*H 9 did not kill at the highest concentration tested, 100 μg/ml.

In vitro microbial inhibition tests. Appropriate volumes of stock acetone solutions of the test compounds were added to tubes of melted, sterile nutrient agar (for bacteria) and malt-yeast extract-agar (for fungi) to give the desired final concentration. After being mixed into the media, individual samples were poured into sterile, disposable, polystyrene plates and allowed to harden. Duplicate plates were inoculated with bacteria and fungi in separate operations with an Accu-Drop (The Sylvania Co., Orange, N.J.) dispensing apparatus. Approximately 0.02 ml of each culture was simultaneously dispensed in uniform droplets on the agar surface. Broth cultures

(48 hr) of bacteria were used. Fungal inocula were prepared by harvesting the spores from mature agar slants by washing with sterile water, followed by filtration through sterile gauze. Inoculated bacterial plates were incubated for a minimum of 72 hr at 30 C, whereas the fungal plates were incubated for at least 5 days at 30 C. (The plates were observed after 2 days for growth of *Rhizopus nigricans*, and, if growth had occurred, that portion of the agar was excised with a sterile spatula to prevent overgrowth of the entire plate.) Lack of visible growth at the end of these periods was recorded as inhibition of the organism at the concentration of compound under test. The organisms tested are listed in Table 2 and represent a spectrum of interest in industrial preservation.

Inhibition of sulfate-reducing bacteria. Inhibition of *Desulfovibrio desulfuricans* was determined by the procedure recommended by the American Petroleum Institute (1). Growth of sulfate reducers in the bottles was indicated by an intense blackening of the medium.

Slimicidal activity in simulated pulp suspensions. A test substrate of 0.5% ground wood pulp at *p*H 5.5 and at *p*H 8 was used to determine the slimicidal activity. The inoculum consisted of a pooled mixture of organisms implicated in causing paper mill slime. One-day-old broth cultures of *Candida pelliculosa* and *Enterobacter aerogenes* were used. In the case of *Bacillus subtilis*, the broth culture was 5 days old to allow for spore formation. Spores of the two fungi, *Aspergillus terreus* and *Penicillium chrysogenum*, were harvested with cotton swabs from well sporulated malt-yeast-agar slants, by using 10 ml of sterile saline per slant. The final inoculum was prepared by adding 1.0 ml from each of the above sources to 95 ml of physiological saline. A 1.0-ml inoculum of this mixture was added to 100 ml of pulp suspension to which the test compound had been added. After exposure periods of 3, 24, and 48 hr, portions were subcultured into suitable media to determine the biocidal concentration of the test compound. The subcultures were incubated at 30 C for at least 5 days.

Slimicidal activity in headbox pulp suspensions. DBNPA was tested as the solid material (98% active) and as a solution with the solvent, polyethylene glycol, with average molecular weight of 200. Headbox stocks from two mills were used. One mill was producing a wide variety of paper used in printing, publishing, and other graphic arts. These stocks contained sizable percentages of ground wood fiber and additives, such as starch, that provided comparatively high nutrient levels accompanied by slime control problems. The second mill was producing mainly heavy paper and board grades on cylinder machines, but also was operating one small fourdrinier machine to manufacture corrugating board from kraft clippings. The kraft furnish gave an alkaline paper stock and was purposely selected to determine whether *p*H modified the results with the test compound.

TABLE 1. *Solubility of 2,2-dibromo-3-nitrilopropionamide in common solvents*

Solvent	Grams/100 g of solvent	Temp (C)
Acetone	35	25
Benzene	<1.0	20
Dimethyl formamide	120	25
Ethanol	25	20
Polyethylene glycol (mol wt, 200)	120	25
Water	1.5	25

TABLE 2. *Antimicrobial activity of 2,2-dibromo-3-nitrilopropionamide*[a]

Organism	Concn inhibiting growth (μg/ml)
Enterobacter aerogenes, ATCC 13048	100
Bacillus subtilis, ATCC 8473	100
Desulfovibrio desulfuricans, A.P.I. RP 38	10
Escherichia coli, ATCC 11229	100
Pseudomonas aeruginosa, ATCC 8709	100
Pseudomonas aeruginosa, USDA, PRD 10	100
Salmonella typhosa, ATCC 6539	100
Staphylococcus aureus, ATCC 6538	250
Aspergillus terreus, ATCC 10690	100
Candida albicans, ATCC 10231	100
Candida pelliculosa, ATCC 2149	100
Pullularia pullulans, ATCC 9348	100
Rhizopus nigricans, ATCC 6227A	10

[a] The *p*H of bacterial medium was 7.0 to 7.2, and that of the fungal medium was 5.0 to 5.5.

The test method used was similar to that described by King (4). Essentially, the test involves the use of in-mill headbox stock samples (minus any customary mill treatment) to determine the efficacy of experimental slimicidal additions. Volumes (100 ml) of treated stock in shake flasks, along with proper solvent controls, were kept at the mill headbox temperature peculiar to the sample. Efficacy of treatments was evaluated by appropriate bacterial counts after 3 and 24 hr of exposure. A 1-ml inoculum (or a suitable dilution) was spread over tryptone glucose agar (Difco) contained in a petri plate. The colonies were counted after incubation for 48 hr at 35 C. All samples were run in duplicate.

Pertinent properties (Table 3) of the headbox stock samples were determined to make the performance of the test compounds more meaningful. In addition, after exposure, the samples were examined for odor, color, foaming, or *p*H changes that might have been caused by the slimicide.

Slimicidal activity in paper mill trials. A solution of DBNPA was added at several points in the paper-making operation, as recommended by personnel at the particular mill. Slime control was determined by periodic plate counts and by inspection of

TABLE 3. *Headbox stock characteristics*

Determination	Mill 1	Mill 2
Machine temp (C)	25	30
*p*H	4.6	7.8
Oven dry solids (%)	0.61	1.06
Ash (OD[a] solids basis) (%)	21.7	5.0
Bacterial control counts (per ml)		
0-hr	1,100,000	370,000
3-hr	1,020,000	1,300,000
24-hr	21,800,000	27,700,000
Fiber content (%)		
Unbleached sulfite	40	
Chemimechanical	50	
Broke	10	
Unbleached kraft		100

[a] Optical density.

TABLE 4. *Laboratory slimicide test with 0.5% paper pulp*

Test compounds	Concn (μg/ml) to give kill in		
	3 hr	24 hr	48 hr
2,2-Dibromo-3-nitrilopropionamide	50	5	5
2-Bromo-4'-hydroxyacetophenone	50	10	10
2-Butenylene-bis-bromoacetate	100	50	10

the critical surfaces of the paper-making equipment, combined with the judgment of experienced operators.

Slimicidal activity in cooling water trials. DBNPA was evaluated as a slimicide in a slug treatment for cooling tower water. The water circulated from a large, concrete holding pond with a capacity of 882,000 liters. The makeup water (95 liters/min) came from a nearby river. Control bacterial counts were established for the pond water, and DBNPA was then added as a solid powder near the circulating pump. Subsequent bacterial counts gave an indication of the effect of DBNPA in the system.

In a second field trial, DBNPA was tested with both continuous and intermittent addition. The cooling tower system had a capacity of 727,000 liters with a water makeup rate of 950 liters/min. The cooling tower was located in Louisiana, and the test was started in November, 1970, and continued through November, 1971. A polyglycol solution of DBNPA was added to the cooling tower basin with a metering pump. Slimicidal control was determined by periodic plate counts, by inspection of the cooling tower fill, and by feel for slime formation, as judged by an experienced operator.

Soluble oil preservation. DBNPA was added to commercial oil formulations before dilution with water (1 part oil to 40 parts water, as recommended by the manufacturers). An inoculum was prepared by adding 10 ml of 24-hr broth cultures of *E. aerogenes* and *Pseudomonas oleovorans* (two organisms commonly cited in contaminated soluble oils) to 80 ml of the diluted oil emulsion under test. Control bacterial counts were in the order of 10^8 organisms per ml. The oil emulsions were inoculated (5.0 ml of inoculum per 95 ml of emulsion) and tested for viability after 24 hr of exposure by swabbing samples onto brain heart infusion agar with a cotton applicator.

RESULTS

In vitro inhibition tests. Table 2 summarizes the results of inhibitory tests using micro-

TABLE 5. *Effect of 2,2-dibromo-3-nitrilopropionamide on mill pulp samples*

Concn tested[a] (μg/ml)	After 3 hr				After 24 hr			
	Mill 1		Mill 2		Mill 1		Mill 2	
	A[b]	B[c]	A	B	A	B	A	B
0.25	22[d]		6		31	28		
0.50	35	28	38	20	37	45	18	
1.0	56	55	66	56	78	89	40	21
2.5	91	97	95	95	100	100	48	82
5.0	98	100	99	99			100	100
10.0	100		100	100				

[a] 100% active DBNPA basis.
[b] DBNPA added as solid.
[c] DBNPA added from polyethylene glycol (molecular weight, 200) formulation.
[d] Reduction (%) in bacterial count. See Table 3 for control bacterial counts.

TABLE 6. *Slimicidal activity of 2,2-dibromo-3-nitrilopropionamide added to a cooling water pond*

Time of sampling	Hr of exposure	Bacterial count per ml	Percent reduction
Pretreatment			
4/16/68			
Noon		25–35 (× 10^4)	
1 PM		25–50 (× 10^4)	
2 PM		25–50 (× 10^4)	
3 PM		25–50 (× 10^4)	
60 μg/ml added at 3 PM			
Post-treatment			
4 PM	1	300	99.9
5 PM	2	100	99.9+
6 PM	3	10	99.9+
4/17/68			
7 AM	16	300	99.9
8 AM	17	100	99.9+
11 AM	20	300	99.9
4/18/68			
7 AM	40	200	99.9+

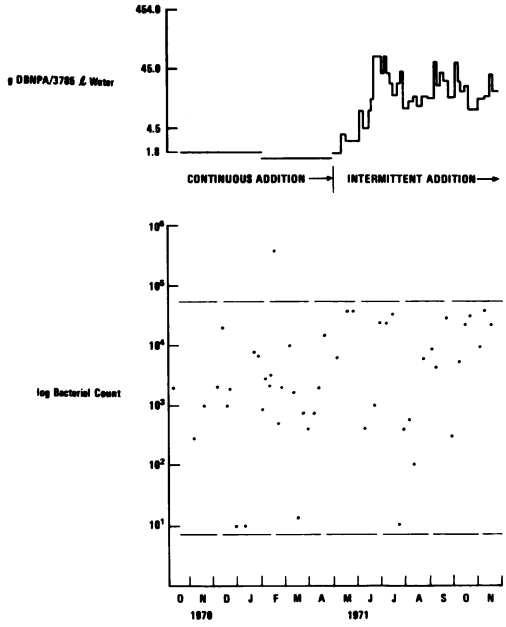

FIG. 1. *Continuous and intermittent addition of 2,2-dibromo-3-nitrilopropionamide (DBNPA) to limit slime formation in a cooling tower system. The rate of DBNPA addition to the cooling tower was correlated with bacterial counts per milliliter made weekly during the treatment period.*

organisms commonly encountered in industrial practice. DBNPA inhibited the microorganisms tested between 10 and 100 μg/ml.

Slimicidal activity in the presence of pulp. Table 4 shows that DBNPA had activity in the same range as two commercial slimicides that also contain bromine.

Slimicidal activity in headbox pulp suspensions. Table 5 shows that DBNPA was active in the two paper mill systems tested, at both *p*H 4.6 and *p*H 7.8.

These results were substantiated by further tests of headbox stocks from mills producing a wide variety of papers. Also, a field trial carried out over a period of a month at a single mill showed that DBNPA (27 g per 907 kg of paper) gave satisfactory slime control. The compound did not cause any mill problems and did not alter the properties of the paper produced during this trial.

Slimicidal activity in cooling water trial. A slug treatment caused a dramatic reduction in bacterial count within 1 hr following the addition of DBNPA (Table 6). This effect persisted for at least 40 hr, and there were no problems (foaming or *p*H change) that arose subsequent to addition of the compound.

DBNPA was used successfully in a second field trial for 13 months. During this time the bacterial count of the cooling tower system remained within the limits demanded by the operators who had had previous experience upon which to base their judgment (Fig. 1). The compound was used both on a continuous and intermittent basis.

Soluble oil preservation. The effectiveness of DBNPA varied depending upon the nature of the soluble emulsion tested. The oils tested represented samples from five major producers of soluble or cutting oils. DBNPA killed the natural inoculum in these emulsions in a range of 25 to 100 μg/ml within a period of 24 hr.

Since most soluble oil formulations have an alkaline *p*H, the hydrolysis of DBNPA limits its extended activity in these systems.

DISCUSSION

Studies of the rate of kill show that DBNPA is bactericidal and fungicidal over a moderate range of concentrations in 1 to 3 hr. This rate is adequate for controlling bacterial and fungal growth in paper mill and cooling tower systems. However, the rate is too slow for DBNPA to be used as a disinfectant.

The breakdown of DBNPA in the *p*H range from 7 to 9.5 may limit its use in single-dose applications where extended antimicrobial activity is mandatory. For example, a single addition can be used to effect bacterial reduction in soluble oil emulsions. However, these emulsions usually have an alkaline *p*H, and DBNPA may fail to protect against repeated bacterial insults within a period of 3 or 4 days.

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