

The Prevention of Fouling with Toxics

The common antifouling paints contain copper, mercury, or arsenic compounds in various combinations. It is the toxicity of these materials which prevents fouling. Toxic action implies that some ingredient of the paint is poisonous to the organisms and must either repel their larvae at the time of attachment or kill them before they can attach permanently and grow. Many investigators have recognized that the toxic must be free to dissolve from the paint in order to be absorbed by the attaching organism.

Copper sheathing was the first extensively used antifouling surface. The first well authenticated application of metallic copper sheathing was in 1763 (55), and this early experiment is described in Chapter 11. It was clearly shown by Sir Humphry Davy (16, 17) that when metallic copper is coupled to zinc or iron it fails to prevent fouling. When so coupled, the copper does not dissolve in sea water, and no toxic solution is present to kill or repel the organisms.

The first authentic record of the inclusion of a toxic in a paint in order to prevent fouling is a patent issued to William Beale in 1625 (5). Mallet (34) patented an antifouling paint in 1841 in which slightly soluble coatings of poisonous materials were applied. These paints failed because he was unable to control the solution rate. Young (71) clearly stated the dilemma which confronts the formulator of an antifouling paint, which is that an insoluble toxic will be ineffective and a soluble toxic will be washed away to leave an exhausted surface. This dilemma has been resolved by learning how to produce paints from which the toxic dissolves just fast enough to prevent fouling, yet slowly enough to last for several years.

The evaluation of the toxic theory of antifouling action requires investigation of the following questions:

1. Are the salts of heavy metals commonly used in antifouling paints really toxic to fouling organisms? What concentrations are lethal? How do they act?
2. Do these metallic salts dissolve in sea water, and is the rate of solution from the paint adequate to maintain a lethal concentration at the paint surface?
3. Is the prevention of fouling by toxic antifouling paints related to the loss of toxics from the paint?

Later chapters of the book will be devoted to some of these questions, but a brief review of the salient points will be presented here so that an evaluation of the toxic theory of antifouling action can be made.

THE ACTION OF TOXICS ON ORGANISMS

Several metallic salts have been reported to be toxic to marine organisms or to their larvae. The concentration at which they are lethal depends both upon the metal tested and upon the organism used. The toxicities of various metallic salts, tested in five different ways, are shown in Table 1. Silver, copper, and mercury are rated as highly toxic in all of these tests. Among these three metals, both copper and mercury have proved very effective in antifouling paints. Silver has not been extensively tested, probably because of its excessive cost.

These tests all rate copper as being somewhat more effective than mercury. This experience has not been universally confirmed when salts of these metals are used in antifouling paints. Adamson (1, 3) found that mercury was somewhat more effective than copper, and Pyefinch and Mott (47) found mercury about twice as toxic as copper to the free-swimming cyprid larvae. In general, however, these two metals and their salts have consistently given good results in antifouling paints.

In addition to copper and mercury, salts of zinc, iron, arsenic, and lead have been commonly used in the formulation of paints. Among these, zinc shows evidence of some toxicity but appears to be only about one-fifth as toxic as copper or mercury. The remainder of these salts are even less toxic than zinc.

An example of the effectiveness of various materials in antifouling paints may be taken from an experiment performed by the Naval Research Laboratory. Ten pigments were added to a matrix to give the same volume loading, and the resulting paints were exposed at Miami Beach, Florida. All of the copper and mercury pigments were effective for periods of ten to fifteen months. The paints pigmented with iron powder, zinc dust, zinc oxide, Celite, and Bentonite were more than 50 per cent covered with fouling during the first or second month of exposure. These results, summarized in Table 2, are in complete agreement with the toxicity results quoted above.

TABLE 1. Comparative Toxicity to Marine Fouling Organisms of Various Metals in Sea Water Solutions

Metal Tested	Compound	Clarke ¹ Lethal to Barnacles		Riley ² Mussel Attachment		Riley ³ Lethal to Bugula		Riley ⁴ Survival Time Bugula		Parker ⁵ Fouling on Metal 6 weeks
		2 days mg/l.	5 days mg/l.	50% mg/l.	All mg/l.	2 hr. mg/l.	5 hr. mg/l.	0.5 mg/l. hours	1.0 mg/l. hours	
Silver	Ag ₂ SO ₄	0.4	0.2	0.2	0.5	0.1		0.4	0.4	
Copper	Basic Cupric Carbonate	0.41								
	CuCl ₂			0.7	1.0	0.5+	< 0.5	3.7		
	CuCitrate	0.60								
	CuTartrate Metal	0.58	0.17							0
Mercury	HgCl ₂	1.0	0.5	0.9	2.0	1.0	0.5	4.0	2.0	
Manganese	KMnO ₄ MnSO ₄			0.9 8.0	2.0					
						10 ⁺⁺	10 ⁺	N/2		
Zinc	Zn(NO ₃) ₂ Metal	32	8	5.0	15.0	10 ⁺	5		22.5	0.2
Iron	FeSO ₄ Metal			5.0						10
Arsenic	As ₂ O ₃ Alkaline			13	40	10 ⁺⁺	10 ⁺	N/2	40	
Tin	SnCl ₂ Metal			15						6
Cadmium	CdSO ₄			30	50					
Chromium	K ₂ Cr ₂ O ₇			20		10 ⁺⁺	10 ⁺	FN	FN	
Lead	PbSO ₄ Metal			40						10
Aluminum	Metal									10

¹ References 13, 14. Table gives the concentration of the metal used which killed all barnacles tested in the indicated period.

² Reference 50. Table gives the concentration which prevents the attachment of 50% and 100% of the mussels tested.

³ Reference 50. Table gives the concentration which kills all *Bugula* tested in two and five hours.

⁴ Reference 50. Table gives the time of survival of *Bugula* in solutions containing 0.5 and 1.0 mg/l. Test conducted for 48 hours; N/2 = half normal at end of test; FN = few normal at end of test.

⁵ Reference 43. Amount of fouling on various metals after 6 weeks' exposure in Eel Pond, Woods Hole, Mass. 0 = no fouling; 10 = completely fouled.

Arsenic has been used frequently in antifouling paints but the results have generally been negative (1, 2, 20). Both arsenic and lead are materials

TABLE 2. Times for the Attachment of Fouling to Paints Containing Various Pigments

Pigment Used ¹	Time in Months to Fall to a Fouling Resistance Rating ² of		
	90%	80%	50%
Cu ₂ O	11	12	13
Copper flake	14	14	15
Copper powder	13	15	—
HgO	12	12	15
Hg ₂ Cl ₂	4-8	10-13	—
Iron powder	1	1	1
Zinc dust	1	1	1
ZnO	1	1	2
Celite	1	1	2
Bentonite	1	1	1

¹ At a pigment loading of 12% by weight in a vehicle consisting of 87% rosin, 12% methyl abietate, 1% Pliolite by weight.

² Fouling resistance as used here and later in this chapter is measured on 8"×10" panels (500 sq. cm. per side) and is defined as follows: A rating of 95 means that larvae have attached, but no forms are matured. Solitary adult organisms reduce the fouling resistance below 95% one per cent for each individual; colonial forms are judged on the per cent surface area covered. Thus a panel on which five barnacles are growing rates 90%. If 10% of the area is covered with encrusting bryozoans, and five barnacles are present, the panel rates 80%, etc.

which one might expect to be effective toxics because of their action on human beings. Metallic lead probably fails to prevent fouling because a very insoluble deposit forms on its surface when exposed in sea water. Arsenic (As₂O₃) was found to be ineffective against barnacles by Harris (20). Wehmhoff, Jordan, and Knight (61) found arsenic trioxide ineffective in hot plastic paints, but Paris Green was said to be an effective toxic. Adamson (1) states that arsenic trioxide and orpiment were tried and gave negative results, and Paris Green alone did not prevent fouling. Harris (20) attributes the low effectiveness of Paris Green to its small content of copper.

Copper, mercury, and zinc are also toxic to the bacteria which form the slime film on a surface submerged in the sea. Their toxicity was tested on pure cultures of these periphytic bacteria by placing the cells in solutions containing various amounts of the toxic. The per cent of the strains which were able to live when transferred after 3 to

4 hours, and after 48 hours, to a nontoxic nutrient broth, are shown in Figure 1. Mercury, at a concentration of 2 mg/l, was shown to kill all of the types tested during 48 hours (59). After 3 to 4 hours, however, 39 per cent of the bacteria in a solution containing 1.0 mg/l were viable (70). Lower concentrations permitted survival of more of the strains, but mercury concentrations as low as 0.002 mg/l still killed about 10 per cent of them in 48 hours. Copper was found to be somewhat less toxic and to act more slowly than mercury. Concentrations of 0.006 mg/l were lethal for some strains; others were able to survive even in concentrations of 6.3 mg. per liter. Waksman, Johnstone, and Carey (57) have isolated bacteria which are able to survive in a medium containing as much as 100 mg. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per liter. Zinc was only about half as toxic as similar concentrations of copper (70).

Differences in the relative resistance of different organisms to the same toxics may also be demonstrated. Weiss (62) found that barnacles were the most resistant forms attaching to antifouling paints at Miami Beach, Florida. Of 662 antifouling paint surfaces studied, 652 ultimately became fouled. The first forms to attach to these paints were as follows:

Barnacles	67% of paints
Encrusting bryozoa	18% of paints
Hydroids	9% of paints
Bugula	5% of paints
Tunicates	1% of paints
Tube worms	0.1% of paints
Sponges	0.1% of paints

Among the barnacles, *Balanus amphitrite niveus* was found to be most resistant, followed by *B. improvisus* and *B. eburneus*. The encrusting bryozoan, *Watersipora cucullata*, was actually more resistant to copper poisoning than were any of the barnacles. The secondary position of encrusting bryozoa may be related to the fact that the occurrence of *Watersipora cucullata* is seasonal, while barnacles are found throughout the year.

British investigators have similarly studied the attachment of various forms, and present their results in terms of sensitivity factors which they use in a method of rating antifouling paints (46). Their results are given in Table 3 of Chapter 20. They found the hydroid *Tubularia* and several of the algae, including the film-forming diatoms, more resistant than the barnacle.

This brief review of investigations of the toxicity of metallic poisons to common fouling organisms indicates that copper and mercury solutions are lethal, and that paints containing them

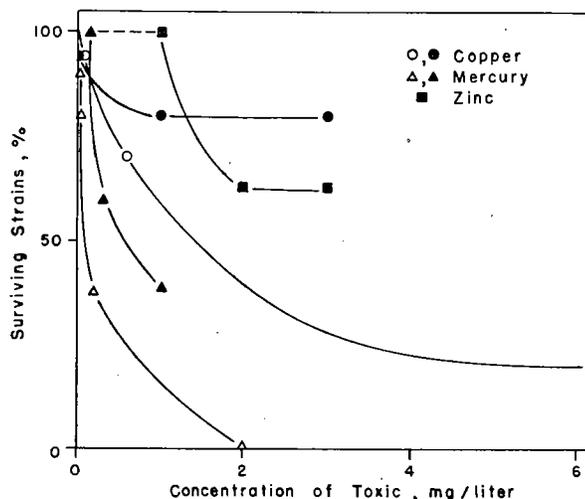


FIGURE 1. The percentage of strains of bacteria resistant to various concentrations of toxics. Solid symbols show per cent of strains surviving after 3 to 4 hours' exposure (70); open symbols, after 48 hours' exposure (59).

may be effective in preventing the attachment of fouling. The same paints containing other materials are not effective. The toxicity of copper and mercury will be discussed in some detail.

The Toxicity of Copper Solutions

The effects of copper solutions on barnacles have been studied by Clarke (13, 14) and by Pyefinch and Mott (47). These investigations have included all stages of development of the barnacle, from the nauplius to the free-swimming cyprid, attached cyprid, newly metamorphosed and adult barnacles.

Pyefinch and Mott (47) studied the toxicity of copper solutions (made with $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) to barnacle cyprids. The results of two of their experiments, given in Figure 2, show that the cyprids remained active for about one day in concentrations of 0.5 mg. copper per liter, and for longer periods as the concentration was decreased. None of the cyprids in solutions containing more than 0.024 mg. copper per liter were able to attach,

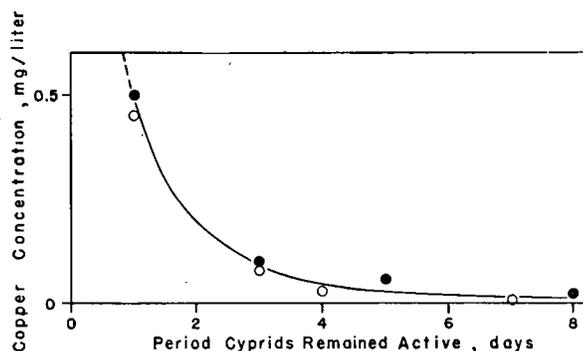


FIGURE 2. Time required to stop the activity of free-swimming cyprid larvae in copper solutions of various concentrations. Data from Pyefinch and Mott (47, Table 2).

TABLE 3. Survival of Barnacle Cyprids in Sea Water to Which Various Concentrations of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ Were Added. The Values Have Been Estimated from Pyefinch and Mott (47, Figure 1)

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ Added mg. Cu/l.	50% Survival Time hours
0.9	24
1.0	8.4
2.0	6.3
3.9	6.0
7.0	2.6

though the cyprids in the control vessels, to which no copper was added, attached in 4 to 7 days. Other experiments by the same authors indicated that the loss of action was not an indication of the death of the organism. When cyprids were transferred to fresh, nontoxic sea water after exposures which made them inactive, they were able to recover. Some experiments showing the time of exposure at which only 50 per cent survived are presented in Table 3. In interpreting the results of these experiments, it must be remembered that the solubility of cupric copper in sea water in equilibrium with air is only 0.5 mg. copper per liter. The addition of cupric sulfate reduces the pH of the solution, thereby increasing the solubility of

TABLE 4. Effect of Different Concentrations of Cupric Citrate in Preventing the Metamorphosis of Barnacle Cyprids (probably *Balanus improvisus*) Attached to Glass Slides

Concentration mg. Cu/liter	No. of Cyprids Exposed	Number of Cyprids Which Metamorphosed
116	6	2 (moult incomplete)
58	6	3
23	4	4
9.7	3	3
4.9	2	2
0.93	11	11
0.47	9	9
0.23	5	5
0.12	9	9

the copper. The amount of cupric ions in solution, however, is probably much less than is indicated by the amount added (cf. Chapter 15).

Following the attachment of the cyprid larvae, metamorphosis to the young barnacle occurs in a period of a few hours. The toxicity of copper during this interval was investigated by Clarke (13, 14), by immersing the freshly attached cyprids, probably *Balanus improvisus*, in solutions of copper citrate.¹ All of the cyprids were able to metamorphose in concentrations as high as 23 mg. of copper per liter. Some of the cyprids metamorphosed even in a solution containing 116 mg. of copper per liter, though in these cases the moult

¹ Cupric citrate forms a complex ion which is very soluble in sea water. Its solubility is not, therefore, limited to 0.5 mg/l., as that of inorganic cupric salts. As will be shown later, Clarke found the toxicity of cupric citrate to be substantially the same as that of other copper salts.

was incomplete. These data are summarized in Table 4.

Similar results were obtained by Pyefinch and Mott, who described the results in terms of five stages of the processes of metamorphosis. These five stages are:

- Cyprid just attached, body of larva projecting up from the substratum.
- Body of larva lying flat on the substratum, anterior end slightly flattened, tissues within showing a groove anteriorly.
- Form of barnacle apparent, cyprid shell not yet shed.
- The cyprid shell has been shed.
- Calcification has begun, the boundaries of the limy plates characteristic of the adult gradually become more distinct.

Even concentrations of 10 mg. per liter were unable to prevent metamorphosis to stage D. The calcification of the shell was retarded in solutions containing only 0.01 mg. per liter. Their data are reproduced in Table 5.

The resistance of newly metamorphosed barnacles to copper poisoning was also investi-

TABLE 5. Effect of Copper on the Metamorphosis of Barnacles

Period of Exposure to Copper	Copper Concentration		Numbers of Barnacles at Stages:			
	Theoretical mg/l.	Measured mg/l.	B	C	D	E
39 hours	Control		0	0	0	15
	0.01	0.003	0	0	17	0 ¹
	0.05	0.029	0	0	16	0 ¹
	0.1	0.092	1	1	17	0
	0.5	0.463	0	1	17	0
	1.0	0.898	0	0	19	0
33.5 hours	1.0		0	0	5	0
	2.5		0	0	4	0
	5.0		0	1	11	0
	7.5		4	0	4	0
	10.0		0	0	6	0
	Control		0	0	0	3

¹ Calcification begins when transferred to fresh sea water.

gated by Clarke and by Pyefinch and Mott. Their results, which are presented in Tables 6 and 7, show that concentrations such as might be expected at the surface of copper paints are lethal in a few days' time. Clarke found, for example, that a concentration of 0.47 mg. copper per liter killed the young barnacles in five days. Lower concentrations required a longer period. These results indicate that the cyprid may be able to attach and metamorphose in toxic solutions which would prevent the survival and growth to the adult form.

Even adult barnacles are sensitive to copper poisoning. Clarke studied several compounds of copper and found that all of them were approximately equally toxic to adult *Balanus balanoides* and *B. eburneus*. Some of his data are included in Table 1 of this chapter. Clarke concluded from his experiments with adult barnacles that the rate of action of cupric citrate is directly proportional to its concentration. This is shown graphically in Figure 3, which relates the reciprocal of the killing time to the concentration of cupric citrate. It was found that whereas a concentration of one mg. of copper per liter was lethal in two days, ten days were required to produce the same effect if the concentration was 0.2 mg. of copper per liter. In these investigations, therefore, no single concentration was found which could be called the "lethal concentration."

Riley (50) has observed that the death rate of barnacles attached to borderline paints is greater

TABLE 6. Relation of Killing Time to Concentration of Cupric Citrate in Barnacles (probably *Balanus improvisus*) Which Had Just Completed Metamorphosis

Concentration mg. Cu/liter	Killing Time Days
9.7	1
4.9	1-2
0.97	3
0.93	3-4
0.47	5
0.23	5
0.20	7
0.12	10

TABLE 7. Effect of Copper on Young Barnacles

Average Cu Concn.	Condition at 12 Days	Condition at 25 Days
Control	Generally active	
0.096	Generally active	Dead
0.19	Many gaping, some sensitive to touch	Dead
0.25	Quiescent, hardly sensitive to touch	Dead

than the death rate of those attached to controls or to less effective paints. These results suggest that, even after attachment, the fouling organisms can be killed by toxics dissolved from the paint.

In an investigation of the causes of the lethal action, Clarke (13, 14) found that the copper was concentrated severalfold in the living tissues of adult barnacles and mussels. No accumulation was found in the shells of the barnacles. No specific internal concentration was found which caused the death of the organism; some died when only 0.19 mg. of copper per gram dry weight was contained in the soft tissue, yet other living barnacles contained as much as 1.09 mg. per gram dry weight. It was observed that the barnacle was

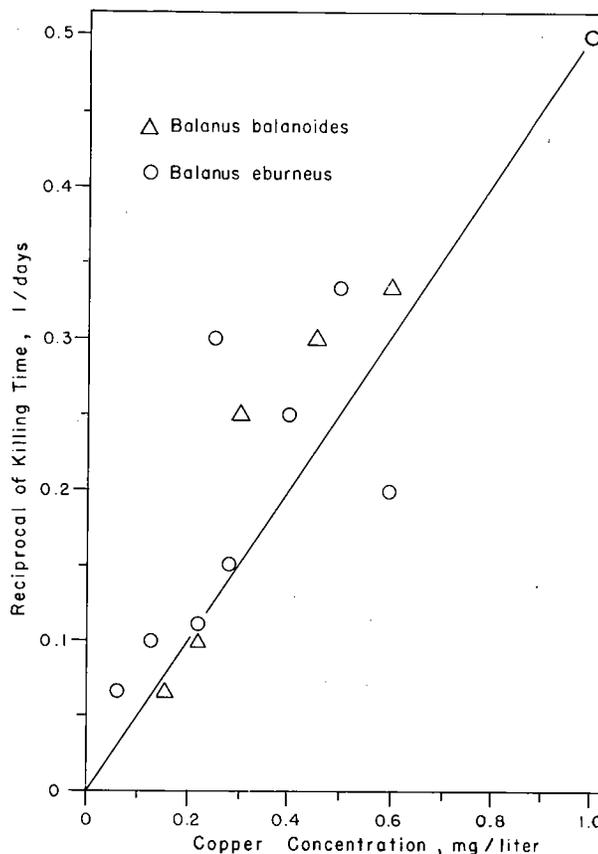


FIGURE 3. Rate of lethal action of cupric citrate on adult barnacles. Clarke (14).

able to excrete considerable amounts of the toxic when replaced in normal sea water. Clarke concluded that the entrance of copper into the tissues of the barnacle is influenced by the metabolic reactions of the living organisms, and that the action of copper is not so much the direct destruction of some tissue or vital material as a general retardation of life processes.

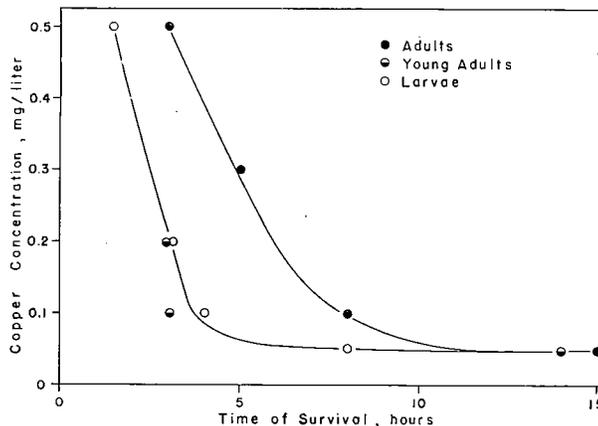


FIGURE 4. Survival of *Bugula turrita* in copper solutions. Redrawn after Riley (50).

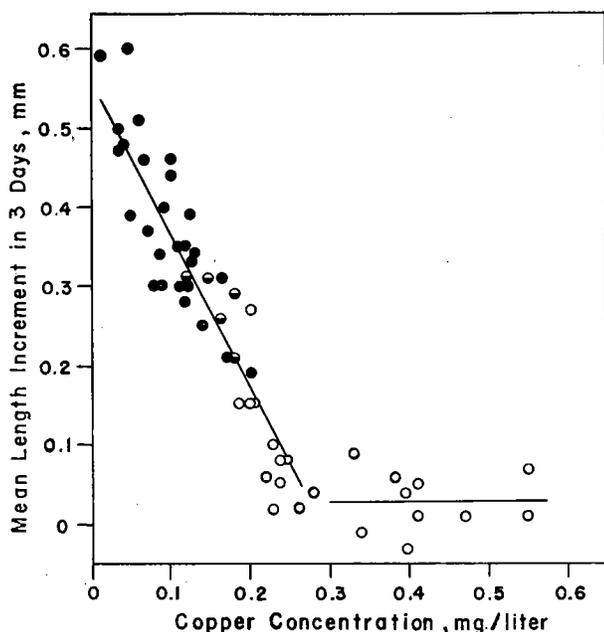


FIGURE 5. Growth of *Bugula* in three days, in relation to copper concentration. ● Fully developed polypides, ◐ partly formed polypides, ○ no differentiation of polypides. Miller (39).

The resistance of larvae and adults of *Bugula* to copper poisoning has been studied by Riley (50) and by Miller (39, 40). Riley's data on the survival of larvae and adults of *Bugula turrita* are given in

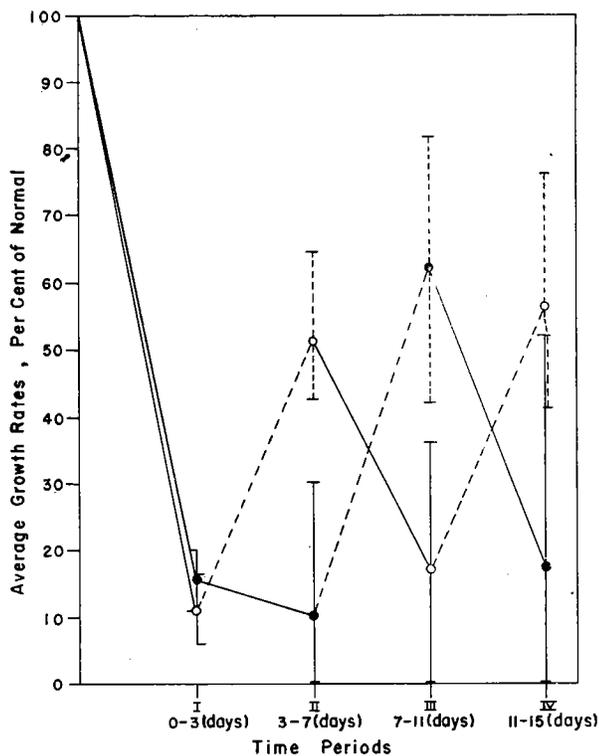


FIGURE 6. Growth rate of *Bugula* during and after exposure to sublethal copper concentrations. Solid lines—growth during exposure to copper solution; dashed lines—growth in fresh sea water; vertical lines show variation. Miller (39).

Figure 4, and show that the adult *Bugula* is more resistant to copper than the larvae or young adults. Miller's results on the growth of *Bugula neritina* are given in Figure 5, and show that the growth of adult *Bugula* and the formation of polypides are both retarded as the copper concentration is increased. Both of these sets of data indicate that concentrations of 0.3 mg. of copper per liter or less are lethal or stop the growth of these fouling forms.

Riley found that the attachment of the larvae of *Bugula* is hastened in copper solutions containing from 0.07 to 0.2 mg. per liter. The majority of larvae in these solutions attached after 3 to 4 hours, while those in solutions containing 0.04 mg/liter or less attached several hours later. Copper concentrations greater than 0.04 mg. per liter or more inhibited the liberation of larvae, but complete suppression does not occur even in concentrations of 0.07 to 0.2 mg/liter which ultimately kill both the larvae and adults.

Intermittent exposures of *Bugula* to sub-lethal doses of copper retards their growth, but does not have an effect equivalent to continuous exposure. Riley found that six or more successive exposures, each of which was calculated to be equivalent to one quarter the lethal treatment, were required to kill *Bugula*. Miller (39) exposed *Bugula* to sub-lethal concentrations of copper which depressed the growth rate. When replaced in fresh sea water the growth rate increased, but did not reach the rate found for the controls which had not been exposed to copper. His results are given in Figure 6. These results suggest that the *Bugula* are able to excrete copper when replaced in fresh sea water. This conclusion agrees with that of Clarke on barnacles, and with Riley's results on the excretion of mercury by *Bugula* which are given below.

The mechanism by which copper exerts its lethal effect is not clearly elucidated by these experiments. Jones (26) has reviewed the theories on the mechanism of toxic action of metals and divides them into two main groups; namely, 1) the metal retards vital processes by inactivating essential enzymes, and 2) it may act more directly by precipitating cytoplasmic proteins as metallic proteinate. The mechanism of the toxic action of copper in preventing fouling deserves further study.

The Toxicity of Mercury Solutions

Various data on the toxicity of mercury solutions were included in Table 1 and suggested that mercury is somewhat less toxic, weight for weight,

than copper to adult barnacles, mussels, and *Bugula*. Pyefinch and Mott (47) found that mercury was somewhat more toxic than copper to the free-swimming larvae of barnacles. Some of their data on the survival times of barnacle cyprid larvae in mercury solutions are reproduced in Figure 7.

Riley (50) has studied the reactions of *Bugula* to mercury solutions and found that the rate of accumulation in the tissues is proportional to the concentration of mercury in solution up to about 0.5 mg/l. The amount accumulated was estimated both by an analysis of the amount removed from solution and of the amount absorbed by the organism. The amount removed from solution was always somewhat greater than the amount recovered in the tissues, which was attributed to the excretion of mercury in a form which was not measured by the analytical technique.

Riley used two criteria for the death of the organisms exposed to mercury solutions. One of

TABLE 8. Mercury Present in Tissues of *Bugula* at Time of Death Estimated by Complete Loss of Motion or Failure to Recover When Transferred to Fresh Sea Water

Concentration of Hg in Solution	No Recovery		Loss of Motion	
	Time	Hg. in Tissues	Time	Hg. in Tissues
mg/l.	hours	mg/g dry wt.	hours	mg/g dry wt.
1.0	1.5	0.62	2.5	0.94
0.5	2.6	0.67	3.75	0.83
0.3	4.4	0.71	7	0.84
0.1	14	0.68	24	0.74
0.05	28	0.66	36	0.86

these was the failure of the organism to recover when transferred to fresh sea water; the other was the complete loss of motion. The rate of accumulation of mercury during the period required to observe these two was also found to be approximately proportional to the mercury concentration as shown by Figure 8. The total amount accumulated was in both cases very nearly constant. An average of about 0.67 mg/gram dry weight of tissues was required to prevent recovery, and an average of about 0.84 mg/gram dry weight was required to cause complete loss of motion, regardless of the concentration of mercury in the test solution used. Riley's results, showing the times necessary to produce these effects, and the amounts of mercury accumulated, are given in Table 8.

Riley concluded that: 1) the amount of toxic present in the tissues at the time of death is constant regardless of the concentration in the exposure bath; 2) the rate of accumulation of mercury is a linear function of the concentration of the

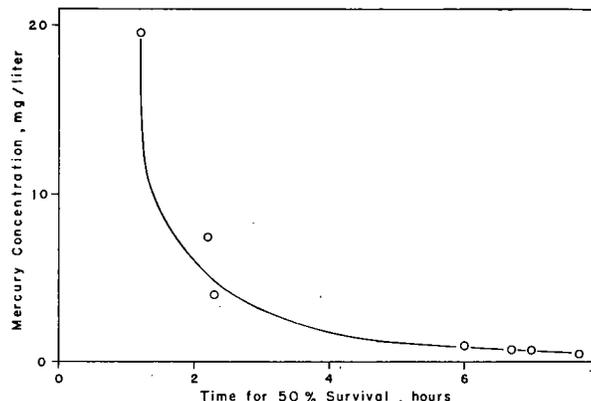


FIGURE 7. Survival of free-swimming barnacle cyprid larvae in sea water containing various amounts of mercuric chloride. Redrawn after Pyefinch & Mott (47).

toxic solution; 3) it follows from 1 and 2 that the rate of action of the toxic is directly proportional to its concentration, except at high concentrations where an avoidance reaction was observed; 4) excretion of mercury occurs both during and after exposure. The total amount excreted, subsequent to sub-lethal exposures, is roughly proportional to the amount of toxic accumulated. Most of it is excreted in combined form. It is excreted rapidly when the tissues have absorbed more than half the lethal dosage. Below this content it is removed slowly.

The Toxicity of Combined Copper and Mercury Solutions

It has been frequently suggested that mixtures

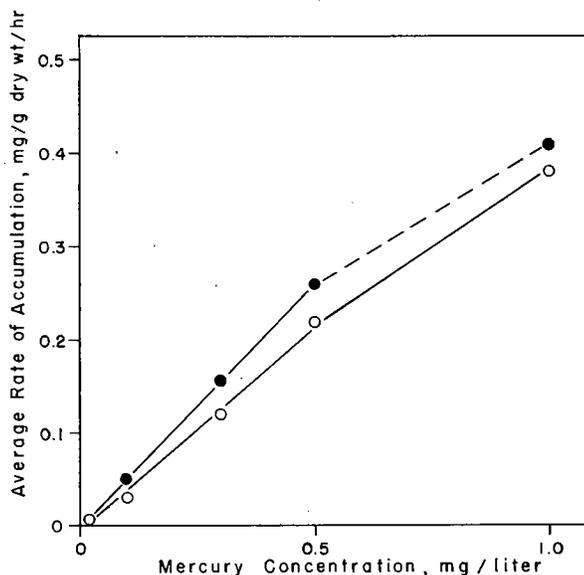


FIGURE 8. Average rate of accumulation of mercury by *Bugula* during the period required for the complete loss of motion, O, and that required to prevent recovery in fresh sea water, ●. Riley (50).

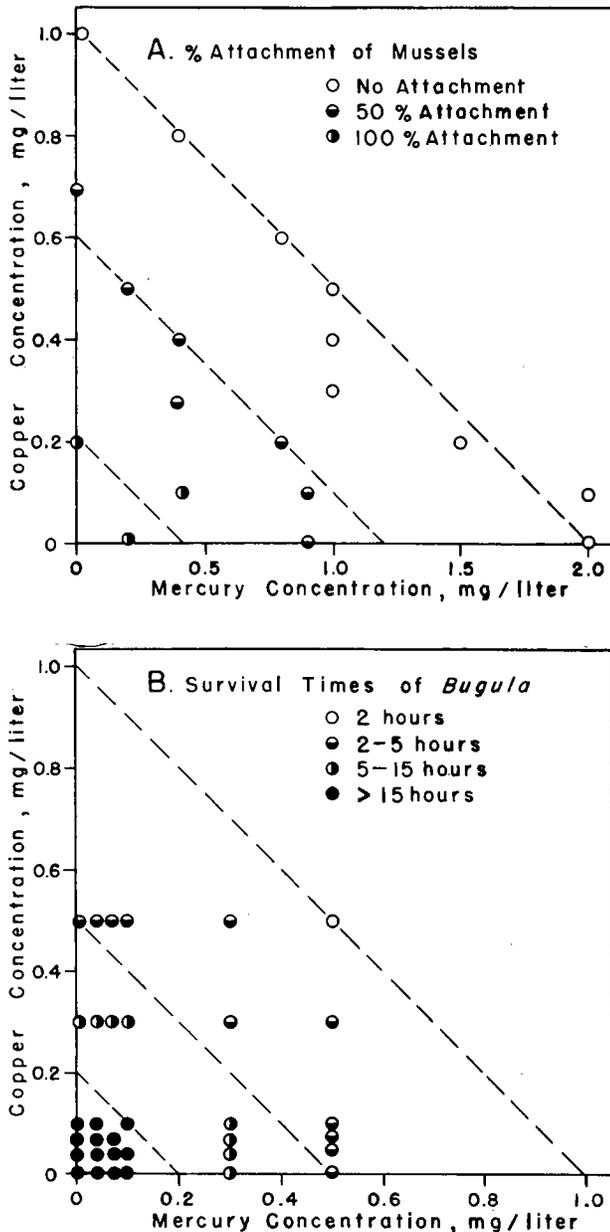


FIGURE 9. The toxicities of solutions containing both copper and mercury. A gives the percentage of mussels able to attach. B gives the survival times of *Bugula*. The dashed lines indicate what might be expected if the toxicities are additive. The amount by which the appropriate points deviate from these lines indicates the deviation from strictly additive results. Redrawn after Riley (50).

of copper and mercury are more effective than either used alone. Investigations of the response of mussels and *Bugula* performed by Riley (50) suggest that the toxicity of mercury and of copper towards these forms is a strictly additive phenomenon proportional to the toxicities of the two ions. Thus, a half lethal dose of copper mixed with a half lethal dose of mercury gives just as toxic a solution as an appropriately stronger dose of either copper or mercury alone. Riley's observations are given in Figure 9, in which mixtures

giving equivalent results are indicated. Pyefinch and Mott (47) studied the effects of copper and mercury mixtures on the survival of free-swimming barnacle larvae. They concluded that the addition of a small amount of mercury was most effective when added to a sub-lethal copper concentration. Hunter (24) and Barnes and Stanbury (9) describe experiments in which the combined action of copper and mercury was greater than would be expected on a strictly additive basis. Further studies of the toxicities of such mixtures would be desirable.

Paints containing various combinations of copper and mercury toxics in the same matrix were exposed at Miami Beach, Florida. The results are shown in Table 9. All of the paints of this series were effective for periods of 13 months or more. The various proportions of mercury and copper had little or no effect on the duration of satisfactory paint performance.

Alexander and Benemelis (4) describe more extensive results with similar paints. Various proportions of mercury and copper toxics were used to produce paints with equal pigment volumes. The substitution of a mercury toxic for an equal volume of copper toxic frequently produced a much more effective paint. Substitution of copper flake or copper powder for part of the cuprous oxide in some formulations also produced more effective paints, and substitution of some zinc

TABLE 9. Times for the Attachment of Fouling to Paints Containing Mixtures of Copper and Mercury Compounds

Pigments and Their Proportions*	Time in Months to Fall to a Fouling Resistance Rating of		
	90%	80%	50%
Cu ₂ O 84, HgO 16	6-8	13	15
Cu ₂ O 67, HgO 33	13	15	16
Cu ₂ O 33, HgO 67	13	14	15
CuF ¹ 84, HgO 16	5	6	16
CuF ¹ 67, HgO 33	12-14	15	16
CuF ¹ 33, HgO 67	15	15	16
CuP ² 84, HgO 16	15	15	16
CuP ² 67, HgO 33	12-14	15	—
CuP ² 33, HgO 67	14	15	16
Cu ₂ O 84, Hg ₂ Cl ₂ 16	11	11	12
Cu ₂ O 67, Hg ₂ Cl ₂ 33	11	12	14
Cu ₂ O 33, Hg ₂ Cl ₂ 67	16	17	—
CuF ¹ 84, Hg ₂ Cl ₂ 16	14	16	—
CuF ¹ 67, Hg ₂ Cl ₂ 33	14	—	—
CuF ¹ 33, Hg ₂ Cl ₂ 67	16	—	—
CuP ² 84, Hg ₂ Cl ₂ 16	14	16	—
CuP ² 67, Hg ₂ Cl ₂ 33	16	—	—
CuP ² 33, Hg ₂ Cl ₂ 67	>16 mo.	—	—

* The compositions of the paints are given in Table 2, which shows the effectiveness of the paints containing only one pigment.

¹ CuF = copper flake.

² CuP = copper powder.

oxide and other nontoxic pigments similarly improved antifouling performance. It is difficult, therefore, to decide whether their results with mixtures of toxics really reflect a synergistic toxic effect, or whether other properties of the paint were primarily affected.

The advantage of mixtures of copper and mercury which have been claimed so often in the past probably can be largely attributed to the fact that the paints were not properly formulated to be satisfactory when only one toxic was used. Differences in the tolerance of organisms to two toxics may

problem. Experiments with continuous exposures of various organisms to chlorine solutions gave the data presented in Table 10 (56). A residual chlorine concentration of 10 parts per million effectively killed all of the organisms tested in 5 days or less. When the concentration of chlorine was one part per million, anemones were able to survive for 15 days but most of the other forms were killed in shorter periods. All but the most resistant organisms are injured when exposed continuously to this concentration. It is, however, sufficiently irritating to cause shelled animals to

TABLE 10. Percentage Survival of Organisms after Continuous Treatment with Chlorinated Sea Water of Various Strengths and for Various Times (56)

Concentration of Chlorine	Animal	Length of Treatment (days)												
		0	1	2	3	4	5	6	7	8	9	12	15	
10 p.p.m.	Anemones	100	100	100	50	0	0							
	Mussels	100	95	65	35	10	0							
	Barnacles	90	20	20	0	2	0							
	Molgula	100	0	0	0	0	0							
	Bugula	100	0	0	0	0	0							
2.5 p.p.m.	Anemones	100	100	100	100	75	60	50		0				
	Mussels	95	95	85	35	20	0	0		0				
	Barnacles	100	50	25	5	0	0	2		0				
	Molgula	100	0	0	0	0	0	0		0				
	Bugula	100		0	0	0	0	0		0				
1 p.p.m.	Anemones	100	100	100	100		100		100		100	100	100	100
	Mussels	100	100	75	65		45		35		50	15	0	0
	Barnacles	100	90	90	50		25		0		0	2	20	0
	Molgula	100	100	100	0		0		0		0	0	0	0

also be important. Weiss (62) found that the bryozoan, *Watersipora cucullata*, is more resistant to copper and more sensitive to mercury than the common barnacles of the Miami region. Clearly, a borderline paint which contains some mercury might give better performance in these waters than a paint which depends solely on copper for its toxicity.

Either toxic alone will give satisfactory long-term performance if the paint is properly formulated. If, however, a paint just fails to be satisfactory, the addition of a small amount of another toxic—mercury added to a copper paint, for example—may be sufficient to make the paint satisfactory. Reformulation of the paint or increasing the content of the original toxic would probably have the same effect.

The Toxicity of Chlorine Solutions

Fouling is frequently troublesome in pipes and conduits supplying sea water on shipboard or for cooling purposes in condensers and equipment ashore. The treatment of sea water with chlorine is a convenient method for dealing with this

close up so that they can survive for several days

These experiments were conducted on the adults, and further investigations indicated that treatment of pipes in which the population had not been allowed to develop was more effective. Thus, continuous treatment of circulating sea water pipe lines with one part per million chlorine completely prevented the growth of fouling organisms at Miami, Florida (56).

The results of intermittent treatment of sea water with chlorine at a concentration of 10 parts per million are sufficient to kill any organisms, except anemones and mussels, exposed to such a solution for only one hour per day. As the duration of immersion in the chlorine solution is increased and the time in fresh sea water correspondingly decreased, the effectiveness of the treatment is improved. The organisms which are capable of closing up, such as mussels and anemones, are able to survive for very long periods with intermittent exposures to chlorine. These results are described in Table 11.

In an extensive test of chlorinating sea water in pipes, a residual chlorine concentration as small as

TABLE 11. Percentage of Survival of Fouling Organisms During and After Periodic Treatment with Chlorinated Sea Water Containing 10 p.p.m. Residual Chlorine (56)

Period of Treatment hours/day	Animals	Length of Treatment (days)										Survival 10 days later†	
		1	2	3	4	5	6	7	8	9	10		
0	Anemones	100	100	100	100	—	100	100	100	100	100	100	100
	Mussels	100	100	100	—	—	100	100	100	100	100	100	100
	Barnacles	100	100	—	85	—	85	—	—	—	—	85	85
	Molgula	100	—	100	—	—	—	—	100	—	—	100	90
	Botryllus	100	—	75	—	—	—	—	75	—	—	75	0
	Bugula	100	—	100	—	—	100	—	—	—	—	50	50
1	Anemones	100	100	100	100	100	100	100	100	100	100	100	100
	Mussels	100	100	100	100	95	95	95	95	95	95	95	95
	Barnacles	100	25	25	10	10	10	10	10	10	10	10	0
	Molgula	75	50	50	5	5	5	5	5	5	5	0	0
	Botryllus	20	20	—	—	10	10	10	0	0	0	0	0
	Bugula	50	—	—	—	0	0	0	0	0	0	0	0
2	Anemones	100	100	100	100	100	100	100	100	100	100	100	100
	Mussels	100	100	100	100	100	100	100	100	100	100	100	100
	Barnacles	100	65	10	10	10	10	10	10	10	10	10	5
	Molgula	75	20	10	10	0	0	0	0	0	0	0	0
	Botryllus	20	10	—	—	0	0	0	0	0	0	0	0
	Bugula	50	—	—	—	0	0	0	0	0	0	0	0
4	Anemones	100	100	100	100	100	100	100	100	100	100	100	100
	Mussels	100	100	100	100	100	100	100	100	100	100	100	100
	Barnacles	100	75	50	10	10	10	7	7	7	5	5	5
	Molgula	75	20	10	10	0	0	0	0	0	0	0	0
	Botryllus	20	0	0	0	0	0	0	0	0	0	0	0
	Bugula	10	—	—	—	0	0	0	0	0	0	0	0
8	Anemones	100	100	100	100	100	100	100	100	100	100	100	100
	Mussels	100	100	100	100	100	100	100	100	100	100	100	100
	Barnacles	65	65	50	10	0	0	0	0	0	0	0	0
	Molgula	75	10	10	10	0	0	0	0	0	0	0	0
	Botryllus	20	—	0	0	0	0	0	0	0	0	0	0
	Bugula	10	—	—	—	0	0	0	0	0	0	0	0
24*	Anemones	100	—	—	—	0	100*	100	100	50	0	—	—
	Mussels	50	—	—	—	0	100*	90	50	10	2	0	0
	Barnacles	25	—	—	—	0	50*	10	0	0	0	—	—
	Molgula	10	—	—	—	0	25*	25	0	0	0	—	—
	Botryllus	0	—	—	—	0	10*	10	0	0	0	—	—
	Bugula	10	—	—	—	0	10*	—	0	0	0	—	—

* The population of this jar was renewed on the 6th day after all the dead organisms had been removed and the jar cleaned.

† The organisms were in running, unchlorinated sea water during this period of 10 days.

0.25 part per million was effective in preventing all fouling growth, but caused accelerated corrosion of steel pipe at high velocities of flow (56).

The Effectiveness of Organic Toxics

Interest in organic toxics has been stimulated by the recent observations that DDT, dichlorodiphenyl-trichloroethane, is effective in preventing the attachment of barnacles when it is incorporated in a paint (18, 35, 54). These investigators point out, however, that DDT is not effective against any of the other common fouling organisms. This sort of observation is almost universally applicable to organic toxics. They are specific in their action, rather than general poisons as are the toxic heavy metals.

The British investigators have studied the toxicity of 91 organic compounds (21, 22). Several were discovered which were toxic to a marine copepod at one thousandth, and to seaweeds at one

hundredth, the lethal concentration of copper as copper sulfate. Many of these toxic compounds were not, however, satisfactory in the paint coatings tested. The investigators found that the following did possess appreciable antifouling properties:

1. Several organic arsenicals, e.g., Diphenyl arsenious oxide, β -Chlorovinyl arsenious oxide, Chlorphenarsazine, 10-Ethyl, 5:10-dihydrophenarsazine.
2. Several derivatives of thiocarbamic acid, e.g., Ferric dimethyl dithiocarbamate, Zinc phenyl dithiocarbamate.
3. Closely related to 2, derivatives of thiourea, e.g., a few thiuram sulfides, though none of these is outstanding.
4. Ethyl bromoacetate (a tear gas).
5. Derris extract.
6. Phenyl-mercury compounds.

Their conclusions from this study were that the organic compound must both be toxic to copepods at a concentration of 10^{-5} grams per ml. and be only poorly soluble in naphtha. These are not the only requirements, but they are almost a *sine qua non* for success.

The toxicity observations of these investigators

effective in preventing fouling when incorporated in a varnish vehicle paint. During World War II, the German Navy also tested organic mercurials, such as methyl mercuric chloride (25). They found that the danger with metallic organic poisons was that they are poisonous to men as well as to marine growths. More complex compounds, not toxic to

TABLE 12. Toxicity of Various Organic Poisons After Harris (21)

Organic Poison	Toxic Conc. (-log ₁₀)
2:4 Di-isobutyl phenol	8.2
Nitroso m-cresol	8.2
4-Nitroso o-cresol	8.2
Chlorophenarsazine	8.0
Tetramethyl thiuram disulfide	8.0?
Di-p-dimethylamino benzophenone	8.0
Ethyl bromoacetate	8.0
Phenyl mercury nitrate	8.0
p-dimethylaminophenyl isothiocyanate	7.0
Pyrethrum (stabilized)	7.0
Diphenyl arsenic acid	6.8
Pentachlorophenol (copper Pyridine complex)	6.8
4-nitroso 1:naphthol	6.8
Trichlorovinyl arsine	6.7
Phenyl mercury acetate	6.7
Acridine	6.6
Pentachlorophenol	6.2
p-dimethylaminophenyl thiocyanate	6.2
Derris extract	6.1
Ferric dimethyl dithiocarbamate	6.0
Zinc phenyl dithiocarbamate	6.0
Di-o-tolyl thiourea	6.0
Tetramethyl thiuram monosulfide	6.0
Tetraethyl thiuram monosulfide	6.0
3-phenyl 5-methyl tetrahydro 1:3:5-thiadiazine 2-thione	6.0
2:4:6 Trichlorophenol	6.0
p-Chlor m-xylene	6.0
Monochlor thymol	6.0
Monochlor isothymol	6.0
Pentachlorophenol (copper salt)	6.0
Pentachlorophenol (mercury salt)	6.0
Aminoza toluene	6.0
Quinoline	6.0
Thiocyano benzthiazole	5.9
Diphenyl arsenious oxide	5.8
$\beta\beta'$ -dichlorovinyl arsenious oxide	5.8
p-nitroso dimethyl aniline	5.8
Benzene azo- β -naphthol (copper complex)	5.7
4:chloro 2:6 dinitro phenol	5.6
Zinc isopropyl xanthate	5.5
Diphenyl thiourea	5.5
Dodecyl thiocyanate	5.5
Phenyl methyl nitrosamine	5.5?

are summarized in Table 12. In this table the toxicity is expressed as a negative logarithm to the base 10 of the concentration required to kill 50 per cent of the test organisms used in 24 hours. This scale is similar to that used for the expression of hydrogen ion concentrations as the pH. Thus, a toxicity index of 6 means that 10^{-6} grams per ml. (1.0 mg/l) were toxic in the test.

Several organic mercury compounds are rated as highly toxic in this list. Similar compounds have been used by Young (72), who has found them very

TABLE 12—(Continued)

Organic Poison	Toxic Conc. (-log ₁₀)
N-N amyl benzyl cyclohexylamine	5.5
Tetraethyl thiuram disulfide	5.4
Phenothiazine	5.4
Carbazole	5.4
p-Dichlorobenzene	5.4
o-cresotic acid	5.4
Nicotine abietate	5.4
Aniline disulfide	5.3
N-methyl benzthiazole thione	5.3
Chloronaphthalene (mixed)	5.3
Salicyl anilide	5.2
β -Chlorovinyl arsenious oxide	5.0
10-Ethyl 5:10 dihydrophenarsazine	5.0
Arsenobenzene	5.0
Isopropyl phenyl carbamate	5.0
Diphenylamine	5.0
p-iodonitrobenzene	5.0
m-dinitrobenzene	5.0
o-Nitroanisole	5.0
o-hydroxy benzene azo β -naphthol	5.0
p-Nitrobenzyl cyanide	5.0
4:Amino 1:2 azonaphthalene	4.8
Phenyl arsenious oxide	4.7
Selenodiphenylamine	4.7
Nitrosophenol	4.7
Phenyl arsenic acid ¹	4.6
p-Bromo acetanilide	4.6
2:4:5 Trichlorophenol	4.6
α -nitronaphthalene	4.5
o-Nitrophenyl arsenic acid	4.0
Copper diphenyl arsenate	4.0
Methyl dimethyl dithiocarbamate	4.0
Phonoztellurine	4.0
p-Chloro acetanilide	4.0
Phenyl arsenic acid ¹	4.0-
p-Aminophenyl arsenic acid	4.0-
p-Amino acetanilide	4.0-
Sulfanilimide	4.0-
p-Dimethylamino azobenzene	4.0-
Benzene azo- β -naphthol	4.0-
p-hydroxy benzene azo β -naphthol (copper complex)	4.0-

¹ Samples from different sources.

the painters, were not effective in preventing fouling.

Pentachlorophenol has been used successfully in preventing fouling in experimental pipe lines. Its toxicity in Table 12 was 6.2 (0.63 mg/l). The times required for 10 and 1 part per million to kill various marine organisms are shown in Table 13. A concentration of 1 mg/l is effective in killing mussels, anemones, barnacles, *Molgula*, and *Bugula* in periods of 1 to 4 days. This concentration permits the growth of slime but almost completely

TABLE 13. Percentage Survival of Organisms After Continuous Treatment with Sea Water Containing Sodium Pentachlorophenate (56)

Concentration Pentachloro- phenate	Animal	Length of Treatment (days)								
		0	1	2	3	4	5	6	7	
10 mg/l.	Anemone	100	0	0	0	0	0	0	0	0
	Mussel	100	95	50	10	4	0	0	0	0
	Barnacle	75	25	5	0	0	0	0	0	0
	Molgula	100	0	0	0	0	0	0	0	0
	Bugula	100	0	0	0	0	0	0	0	0
1.0 mg/l.	Anemone	100	100	100	20	0	0	0	0	0
	Mussel	100	100	15	0	0	0	0	0	0
	Barnacle	80	35	10	10	0	0	0	0	0
	Molgula	100	0	0	0	0	0	0	0	0
	Bugula	100	0	0	0	0	0	0	0	0

prevents the development of fouling organisms when injected into water flowing through pipes (56). A concentration of 0.5 part per million was found to permit the growth of tubeworms and mussels, but to weaken them so that velocities greater than 0.5 knot prevent their attachment and growth.

THE PREVENTION OF FOULING WITH PAINTS

This brief review of the toxicity of various materials shows that copper, mercury, and some organic compounds are poisonous in concentrations of about one milligram per liter (one part per million). Experiments with paints designed to prevent fouling by the toxicity of an ingredient show that the toxic must be dissolved from the paint by the sea water in order to be effective. The difficulty in making satisfactory antifouling paints has been in devising formulations which release the toxic at a rate sufficient to provide a lethal concentration at the surface but still slow enough to prevent the rapid and complete exhaustion of the toxic reserve.

Release of Toxic from Paint Films

Proof that the toxic materials of a paint film are liberated into the water in concentrations which exert an effect on the fouling organisms is afforded by both biological and chemical experiments.

Pomerat and Weiss (45) studied the ability of antifouling paints to protect adjacent unpainted areas from fouling. Holidays of various sizes were left on sandblasted glass panels, the remainder of the surface being coated with an antifouling paint. It was found that an effective paint could completely inhibit the attachment of fouling on unpainted circular areas up to about one centimeter in diameter. Figure 10 shows panels which were exposed for 45 days at Tahiti Beach, Florida. On two of the paints the tubeworms, which were prevalent fouling forms at the time, were restricted to the center of the large unpainted circles and to the wide region of the unpainted wedge. The panel on the left fouled on the paint surface, and barnacles and tubeworms have attached on the unpainted areas. Figure 11 shows a close-up of a 10 centimeter circle left in paint 16A, erstwhile standard wood

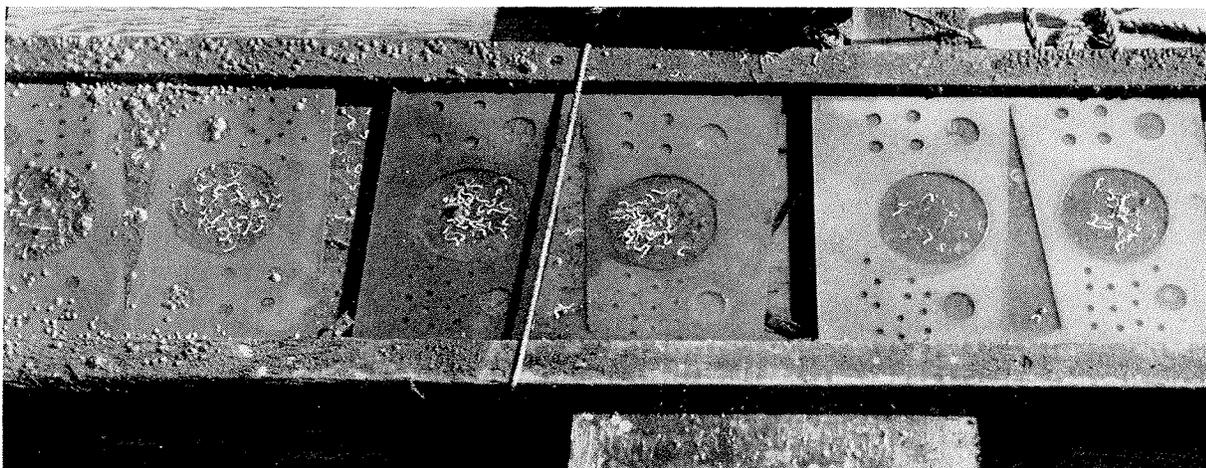


FIGURE 10. Protection of unpainted areas adjacent to proprietary antifouling paints after 45 days' exposure at Tahiti Beach, Florida. After Pomerat and Weiss (45).

bottom paint of the U. S. Navy. In the small unpainted circles, a few attached barnacles can be found. The large circle is almost completely covered with minute attached barnacles. It will be noticed that these barnacles extend practically to the edge of the paint film where, however, they are smaller than those attached in the center of the unpainted area. Weiss (63) described the abnormal development of tunicates which attached adjacent to antifouling paints. These results have been confirmed by Young, using metallic copper sheets

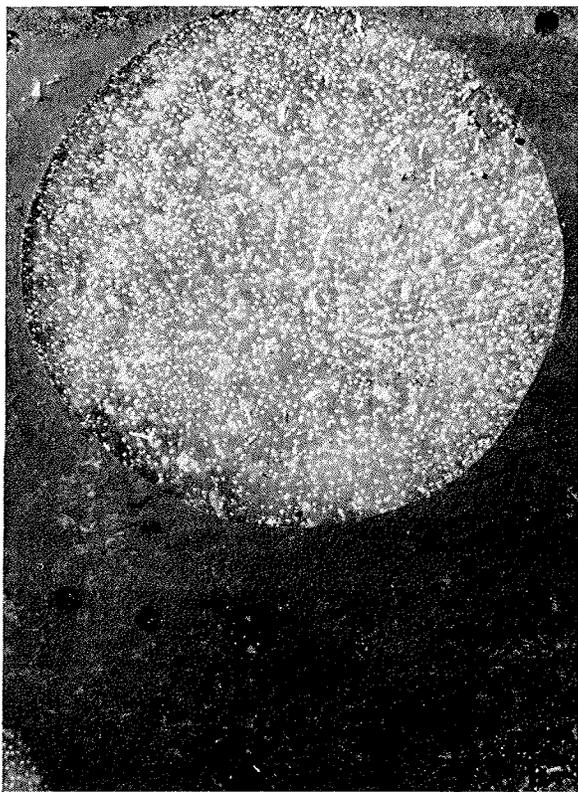


FIGURE 11. Attachment of barnacle larvae to an unpainted area surrounded by a toxic paint after 11 days' immersion at Miami Beach, Florida. Note the greater size of many of the barnacles in the center of the unpainted area. After Pomerat and Weiss (45).

paint surface was demonstrated in a very graphic way by an experiment performed by Miller (39). *Bugula* larvae were permitted to attach to a non-toxic panel. This was then placed perpendicular to an effective cuprous oxide paint surface, and the growth of the *Bugula* stalks was measured. Con-

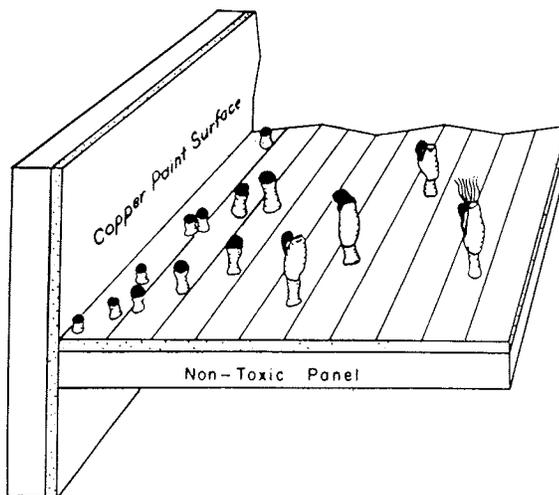


FIGURE 12. The toxic gradient extending from a copper paint surface as shown by the growth of *Bugula*. The *Bugula* figures are camera lucida drawings made after 4 days' exposure, and are twice enlarged in comparison to the millimeter rulings. After Miller (39).

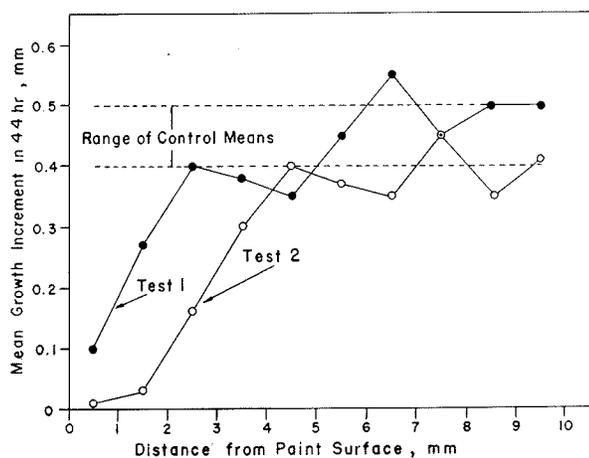


FIGURE 13. Gradients of toxicity of an antifouling paint. (Test 1), paint seasoned 10 weeks and coated with slime film; (Test 2), paint seasoned 12 weeks, slime film removed. After Miller (39).

with wedge shaped Bakelite inserts and copper screening of various sized mesh (72). The effect of metallic copper in preventing fouling on neighboring areas of wood is illustrated in Figure 4, Chapter 21.

These experiments show that the fouling of unpainted areas may be prevented, inhibited, or modified by nearby toxic surfaces. The effect can be explained only by the supposition that the toxics dissolve from the paint and spread over the unpainted areas.

The distribution of toxicity perpendicular to a

controls were grown under similar conditions except that the copper paint was replaced with a non-toxic surface. The organisms close to the paint surface grew very little, compared to the controls, in a period of two days. Miller's results of this experiment are shown in Figures 12 and 13, which show that the effect of toxicity is evident $2\frac{1}{2}$ to $4\frac{1}{2}$ millimeters from the paint surface. It is probable

that this distance is greater than would be observed under normal exposure conditions, where the water is in more active circulation.

The comparative toxicity of paints has been estimated by various biological tests. The most direct of these measures the tendency for fouling organisms or their larvae to attach directly to the paint surface. Tests of the toxicity of leachates from paints, which give additional evidence that the toxics act while in solution, have also been made.

One of the attachment tests described by McLean (38) uses the edible mussel, *Mytilus edulis*. Where available, this mussel can be removed in

TABLE 14. Loss of Weight and of Cuprous Oxide from a Series of Cold Plastic Paints Containing Graded Amounts of Cuprous Oxide during Two Months of Soaking in the Sea

Original Cu ₂ O% ¹	Loss of Cu ₂ O		Loss of Weight mg/cm ²	Fouling Resistance ² %
	mg/cm ²	μg/cm ² /day		
1.4	0.016	0.27	1.30	0
2.5	0.112	1.87	2.84	0
5.3	0.150	2.50	0.97	0
10.5	0.384	6.40	1.25	60
15.6	0.712	11.9	2.32	100
21.8	0.589	9.8	2.27	100
28.1	0.665	11.1	1.17	100
40.5	0.932	15.5	2.04	100

¹ At Tahiti Beach, Florida, after two months' immersion.

large quantities from rocks, piles, buoys, or other submerged surfaces. When they are replaced in sea water they will re-attach to a surface by spinning new byssus threads. McLean studied the attachment of these organisms to various toxic paints. He found that all of the mussels would attach to a plain or sandblasted glass surface. None of the mussels attached to satisfactory antifouling paints, though they moved across them. Observations of the mussels on satisfactory paints showed that the foot was extended and that the byssus might be attached momentarily in order to move, but in general the surface had a distinctly repellent effect which was attributed to its toxicity.

Nelson and Kodet (41) determined the time for the immobilization of a diatom (*Navicula ramocissima*) when placed in washings from a toxic panel. Dilution of the washings permitted continued motility for greater periods of time. The mud snail, *Nassa*, was found to be very sensitive to the toxic, and was killed even when the washings from a panel were diluted in 7.2 liters of water.

The larvae of *Bugula* have been used by Miller (39, 40) and by Riley (50) in studies of the toxicities of various paints. This larva is a free-swimming form which can be collected by placing the gravid adult in a beaker of sea water in the labora-

tory. The larvae are liberated early on the following morning with remarkable regularity, so that experiments may be planned in advance. The larvae were collected and either placed in a cup which was painted on the inside and contained sea water, or in a beaker in which a painted slide was suspended. The number of larvae attached to the paint surface was taken as an indication of the toxicity of the surface. On nontoxic paints all of the larvae were able to attach to the paint and to grow after attachment. On more effective antifouling paints fewer of the larvae attached, and those which did attach grew at a reduced rate, if at all. These experiments show clearly that a toxic paint may act in either of two ways. It may repel the larva so that it never becomes attached to the paint surface, or, if the organism does attach, the toxic may prevent its growth and development to the mature state. These results can be interpreted only on the basis of the toxic or irritant action of some paint ingredient.

Bray (10) investigated the toxicity of solutions of various metallic salts in sea water and compared these results with the toxicity of sea water leachates from small painted panels. He found that the more effective paints gave leachates which were highly toxic to the nauplius larvae of the barnacle.

Various chemical means of measuring the loss of toxic from paint films have been tried. Orton (42) determined the copper and mercury content of various paints after prolonged soaking in the sea when the paints were just beginning to become fouled. He found that the percentage content of copper in these paints was considerably less than the original concentration. He correlated the failure of the paint film with the concentration of toxic remaining in the paint at the time of failure. Edmondson and Ingram (19) also observed that the copper content of paints decreased after prolonged soaking in the sea. It is apparent that in these experiments the toxic materials were preferentially soluble in sea water, and were leached out at a proportionately faster rate than the other paint materials.

The determination of the total weight lost from a paint film during immersion in the sea permits comparison with the data on exfoliation presented in Chapter 13. Numerous experiments have been performed in which panels coated with a known weight of paint of known copper content were immersed in the sea for various times. The weight and analysis after immersion gives the loss of weight and of cuprous oxide. Typical results are given in Table 14, which show that the total loss

TABLE 15. The Leaching Rates of Cold Plastic Paints Containing Graded Amounts of Cuprous Oxide (30)

Cu ₂ O Content ¹	Copper Leaching Rate, $\mu\text{g}/\text{cm}^2/\text{day}$ After Soaking							
	0	1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.	8 mos.
0.4	1.1	1.0 ^f	0.6 ^f	1.3 ^f	0.3 ^f	0.6 ^f	6.1 ^f	0.6 ^f
1.23	2.9	1.2 ^f	0.9 ^f	1.7 ^f	0.6 ^f	0.6 ^f	0.3 ^f	0.9 ^f
2.23	5.0	1.3 ^f	0.9 ^f	3.1 ^f	0.9 ^f	0.9 ^f	1.2 ^f	—
4.64	17.8	3.9 ^f	1.6 ^f	2.4 ^f	1.9 ^f	2.8 ^f	2.5 ^f	1.2 ^f
9.3	25.0	7.5	4.7 ^f	4.4 ^f	1.9 ^f	3.7 ^f	7.1 ^f	3.3 ^f
13.8	27.4	14.1	11.3	10.0	10.5	lost	—	—
19.2	40.6	19.3	11.3	8.8	8.1	10.1	8.0	8.1
24.8	51.5	27.7	12.5	9.5	12.2	12.3	9.8	9.0
30.0	55.1	28.5	13.4	9.7	10.3	13.8	14.4	13.8
36.0	57.4	30.2	11.6	10.9	12.0	14.4	14.7	14.1
50.7	47.2	44.3	21.0	14.2	15.6	20.2	18.7	17.7

¹ As % dry weight of the paint film. The pigment volume was maintained constant by the substitution of magnesium silicate.

^f Paint fouled when exposed the indicated time at Tahiti Beach, Florida.

of weight does not determine the fouling resistance of these paints (31, 32). Their effectiveness is related to the rate of liberation of the copper, which was released from the effective paints at rates of 9.8 to 15.5 micrograms/cm²/day. The weight loss of all of the paints was 1–2 mg/cm² during the two month period, or 0.020–0.039 mg/cm²/day. LaQue has shown that the fouling of copper and copper alloys is also related to the rate of loss of copper (33). These results are discussed in detail in Chapter 21.

The rate of loss of toxics from a paint can also be determined by measuring the amount dissolved in sea water in which a painted panel has been soaked for a short period of time. The method for determining such leaching rates has been worked out for paints containing copper (23, 29, 30, 36, 37, 59) and mercury (49, 51–53). In practically all cases the effectiveness of the paint in preventing fouling is directly related to the leaching rate of the principle toxic ingredient. The method for determining leaching rates is described in Chapter 20.

The leaching rates of all paints which have been investigated change during a period of immersion in the sea. It is necessary, consequently, to determine the leaching rate at frequent intervals in order to determine how it changes with time, and how soon the value decreases below the minimum critical value. Curves showing the changes in leaching rate of several paints after immersion in the

sea are given in Chapter 16. The leaching rate determination is an indication of the toxicity of the paint film only at the time the determination is made. It is of no value in predicting the effectiveness of the paint at some future date.

Fouling in Relation to the Leaching Rate

Considerable data are available to show the correlation between leaching rates and the fouling resistance of the paint. Table 15 shows the leaching rates of a group of eleven paints after various periods of immersion in the sea (30). These paints all had the same vehicle composition, but the cuprous oxide content of the formulation varied from about 50 per cent of the dry weight to less than one per cent. Magnesium silicate was substituted in equal volume for the amount of cuprous oxide removed in every case. The six paints containing the greatest amounts of cuprous oxide were effective in preventing fouling. All of these had copper leaching rates greater than 8 $\mu\text{g}/\text{cm}^2/\text{day}$. The five paints which contained less than 10 per cent cuprous oxide became fouled during the first two months of exposure at Tahiti Beach, Florida, and all of these paints had leaching rates less than 7.1 $\mu\text{g}/\text{cm}^2/\text{day}$ at the time of fouling.

Although the efficiency of a paint is related to the toxic content of the film, an amount of copper which is adequate in one composition may be inadequate in another formulation. Table 16 shows

TABLE 16. Leaching Rates of Paints in Which Various Resins Were Substituted for Rosin in the Navy Department Specification 52 P-61 Type Paints. All of the Paints Contained 52% by Weight Cuprous Oxide in the Dry Film (30)

Resin Used	Copper Leaching Rate, $\mu\text{g}/\text{cm}^2/\text{day}$ After Soaking						
	0	1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.
W. W. Rosin	30.0	18.0	16.2	13.5	14.1	16.2	15.8
Unesterified Albertol #1	33.3	20.4	17.1	13.8	16.5	18.0	18.8
Unesterified Albertol #2	32.1	11.1	10.5	—	9.6	10.8	9.8
Ester Gum	30.3	3.4 ^f	5.5 ^f	4.2 ^f	2.7 ^f	0.9 ^f	0.9 ^f
Esterified Albertol	5.7	0.9 ^f	0.0 ^f	—	3.3 ^f	0.0 ^f	—
Coumarone Indene	25.6	4.0 ^f	2.1 ^f	2.1 ^f	4.2 ^f	—	1.5 ^f

^f Paint fouled when exposed the indicated time at Tahiti Beach, Florida.

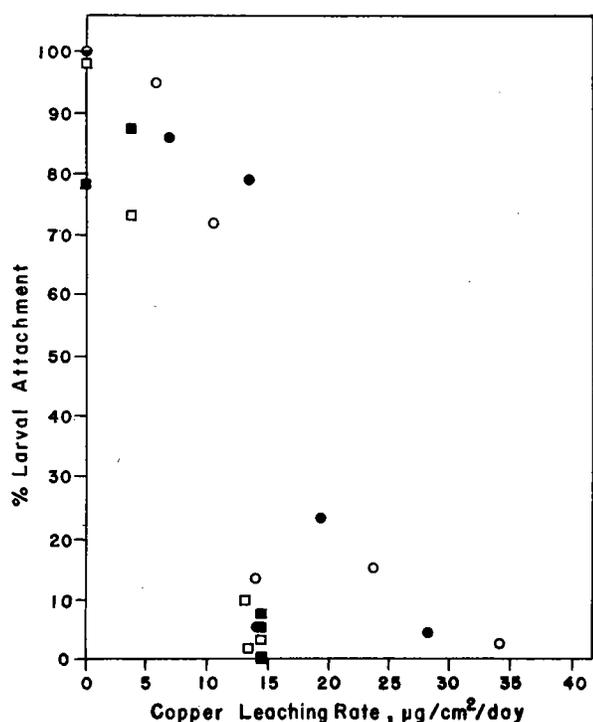


FIGURE 14. Attachment of *Bugula* larvae in relation to copper leaching rate. Filled data points represent paint surfaces coated with slime film. Clear data points represent surfaces wiped before testing. All paints were seasoned for ten weeks in sea water before testing. After Miller (39).

the results of an experiment on six paints, all of which contained the same amount of cuprous oxide. The vehicle was modified by the substitution of various resins for the rosin which was the principal ingredient of the binder of the standard paint. All of the paints which had leaching rates less than 9.6 micrograms/cm²/day became fouled when immersed at Tahiti Beach, Florida. The paints with higher leaching rates resisted fouling during the six months' period of exposure. It is obvious that the cuprous oxide in paints 4, 5, and 6 was not available for dissolution in sea water and consequently did not prevent the fouling of these paints. The mechanisms which enable a paint to maintain an adequate leaching rate will be discussed in Chapter 16.

Miller has studied the attachment of *Bugula* larvae to paints having known leaching rates at the time of the test (39). He found that leaching rates less than 10 micrograms per square centimeter per day permitted larvae to attach in large numbers, and to grow and differentiate. Paints with leaching rates greater than 10 µg/cm²/day allow only a small percentage of the larvae to attach, and they completely inhibit their growth. In a few cases a large number of larvae were found attached to paints having leaching rates between 10 and 15 µg/cm²/day, but these did not in any case develop into colonies. Miller also found that the leaching rate of the paint, rather than its copper content, was the factor which controlled the attachment of *Bugula*. The relation found between the leaching rate and attachment is shown in Figure 14.

The preceding examples describe paints in which cuprous oxide is the principal toxic agent. Experiments have also been conducted on metallic copper paints which indicate that the minimum adequate copper leaching rate for such paints is the same as that for cuprous oxide paints. A typical set of data is given in Table 17. The paints in this series were formulated to contain graded amounts of metallic copper. As long as the copper leaching rate was greater than 10.9 µg/cm²/day, the paints remained free of fouling at Miami Beach, Florida. Thus, the minimum adequate leaching rate of both cuprous oxide and metallic copper antifouling paints appears to be about 10 µg/cm²/day.

The evaluation of the minimum adequate leaching rate has been conducted on more than a hundred different paints using cuprous oxide or metallic copper as the toxic ingredient. The correlation between the leaching rate and fouling resistance of these paints is shown graphically in Figure 15 (27). For this correlation the average of the leaching rates measured at monthly intervals between the second and sixth month of immersion, inclusive, and the fouling resistance after six months of immersion at Miami Beach, Florida, are used.

TABLE 17. Leaching Rates of Paints Containing Graded Amounts of Metallic Copper in a Matrix Containing 50% Rosin and 50% Vinylite (VYHH)

Copper Content ¹ %	Copper Leaching Rates, µg/cm ² /day After Soaking						
	0	1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.
90	256	19.1	11.5	13.8	19.6	16.0	24.2
67.5	190	19.0	11.7	13.3	17.4	15.4	20.2
45	160	21.3	13.6	13.8	16.4	12.6	12.2
31	134	21.5	12.7	13.9	12.5	11.1	16.4
22.5	100	15.0	8.2	10.9 ^f	9.2 ^f	8.8 ^f	5.8 ^f
13.5	67	4.8 ^f	3.3 ^f	—	—	—	—
9	55	1.9 ^f	1.0 ^f	—	—	—	—
4.5	46	0.7 ^f	0.4 ^f	—	—	—	—

¹ As % dry weight of the paint film. No substitution of inert materials was made for the copper removed.

^f Paint fouled when exposed the indicated time at Miami Beach, Florida.

The dividing lines for satisfactory performance are drawn at a leaching rate of $10 \mu\text{g}/\text{cm}^2/\text{day}$ and a fouling rating of 80 per cent. The distribution of fouling resistances and leaching rates may be summarized as follows:

Average Leaching Rate	Fouling Resistance			
	0	1-79%	80-99%	100%
Greater than 10	None	1	18	54
Between 5 and 10	6	3	4	None
Less than 5	25	None	None	None

Of the seventy-three paints which gave average leaching rates greater than $10 \mu\text{g}/\text{cm}^2/\text{day}$, only one was badly fouled; most of them (74%) were completely free of fouling. None of the paints with copper leaching rates less than 10 gave 100 per cent fouling resistance for six months. A few paints which give leaching rates between 5 and $10 \mu\text{g}/\text{cm}^2/\text{day}$ give fairly satisfactory performance. Most of the paints with leaching rates less than 10, however, were completely fouled in six months. The correlation between the leaching rate and fouling resistance was satisfactory for more than 95 per cent of the paints.

Most of the paints either prevented fouling completely or failed completely. This relation might not have been observed if the fouling tests had been performed under less severe conditions than are obtained at Miami Beach. Many paints which foul completely in Florida may appear to be quite satisfactory when exposed during the winter at more northerly locations. In order to obtain correlations as satisfactory as that given above, the fouling tests must be conducted under intense fouling conditions.

As mentioned previously in this chapter, the resistance of different organisms to toxic action may vary widely (46, 62). It is to be expected that similar differences will be found for leaching rates adequate to prevent attachment of fouling organisms. Barnes (6, 7) describes exposures which indicate variation in the leaching rate-attachment correlation as follows:

Critical leaching rate $\mu\text{g}/\text{cm}^2/\text{day}$	Organism affected
20	"Brown Mats" (algal growth)
10	Tubularia & Ectocarpus
9	Balanus
4	Obelia
3	Pomotoceros

Barnes also concluded that a leaching rate of $10 \mu\text{g}/\text{cm}^2/\text{day}$ is adequate to prevent attachment of all animal forms, but that some algae, and particularly the microscopic forms, can attach to paints with higher leaching rates. The algal growth

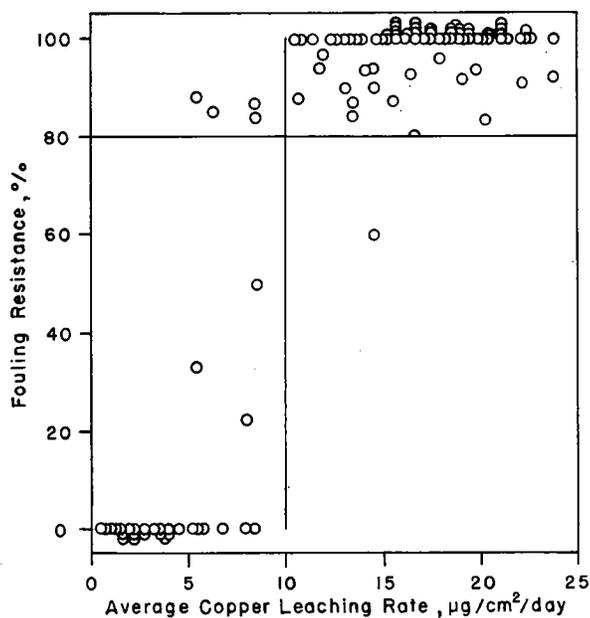


FIGURE 15. The average leaching rates, between the second and sixth months of exposure, compared to the fouling resistance, after 6 months, for several different copper antifouling paints. To permit presentation of all of the data some points for 100% fouling resistance are plotted above the 100% line and for 0% fouling resistance below the correct line. These points should be read as 100% or 0% respectively.

termed "Brown Mats" by the British investigators may be similar to the bacterial and algal growths called "slime films," which are discussed below.

It is apparent that the average leaching rate used in the above correlations is applicable only if the leaching rate of the paint tends to remain nearly constant for the period studied. The leaching rates of many paints decrease gradually during exposure, and their average leaching rate has little meaning. Some examples of paints showing this type of performance are given in Table 18. Although all of these paints have adequate average leaching rates, individual determinations were less than $10 \mu\text{g}/\text{cm}^2/\text{day}$ and the panels became fouled at about the time the leaching rate fell below 10. The average leaching rate consequently shows no correlation with the final fouling resistance. These results show the necessity of measuring leaching rates at intervals during the exposure of the paint, and the desirability of developing a formulation which maintains a leaching rate which is uniformly adequate.

The leaching rate method does not give valid results when used on paints with poor film characteristics. Particles of such paints may be washed off in the water of the leaching bath, where they apparently dissolve at an excessive rate, since they present an increased surface area. High leaching rates may be obtained although the paints

MARINE FOULING AND ITS PREVENTION

TABLE 18. Results of Leaching Rate and Fouling Resistance Tests on Paints Which Do Not Maintain a Steady State Leaching Rate

		Paint Copper Leaching Rate, $\mu\text{g}/\text{cm}^2/\text{day}$ After Soaking					Ave.	F. R.
		2 mos.	3 mos.	4 mos.	5 mos.	6 mos.	L. R.	6 mos.
BK	9	45.0	26.1	14.4	8.9	4.5 ^f	19.8	70
BK	17	39.0	25.0	15.1	7.4	4.5 ^f	18.2	60
PT	2	32.1	11.3	11.3	6.3	0.6 ^f	15.4	33
PT	5	16.8	8.7	12.0 ^f	6.6 ^f	6.2 ^f	10.1	65
PT	8	14.1	8.4	11.1	9.3 ^f	7.2 ^f	10.0	75

^f Fouling resistance less than 80%.

may become fouled. It appears, therefore, that the eroded paint contributes to the leaching rate measurement, but does not contribute to the toxicity of the surface. A series of paints which show this behavior was formulated by modifying the Navy formula 16A. The proportions of metallic brown and cuprous oxide were changed, keeping the pigment volume of the paint constant, as shown in Table 19, which also shows the leaching rates and fouling performance of the paints. All of these formulations had such poor film characteristics that it was impossible to handle them without rubbing off a considerable amount of paint.

During the first four months of exposure only the paints with low leaching rates became fouled. After six months, however, paints 7, 8, and 9 became badly fouled even though the apparent leaching rates were between 25 and 30 $\mu\text{g}/\text{cm}^2/\text{day}$. It is believed that the marked exfoliation of the surface, which became extreme after this time of soaking, contributed to the copper measured in the leaching bath, but did not contribute to the toxicity of the surface. It is notable that this degree of exfoliation was not adequate to prevent the fouling of the surface.

Weith and Turkington (65) observed that effective copper paints rapidly form a grey or green coating of copper salts when exposed in the sea, or in sea water maintained at 40° C. in the laboratory. They suggested that the presence of a layer of relatively insoluble copper salts on the paint

surface might coagulate the proteins of the cement and destroy its adhesion, or that the solution of the deposit would be accelerated if the cement were acidic, or if the carbon dioxide produced in the metabolism of the organism resulted in a local acidic reaction. The deposit might thus prevent fouling without requiring continuous loss of soluble copper from within the paint film. While it is true that metallic copper and most of the effective copper-containing paints do form such deposits, no evidence has been found that this material is dissolved by the attaching organisms. It seems probable that the presence of the green surface deposit is an indication that the paint surface is liberating dissolved copper at a rapid rate. The interaction of cuprous oxide and metallic copper with sea water, and the solubilities of the salts which are formed and which precipitate to form the surface deposit, and its effect on the leaching rate are described in Chapters 15 and 16.

Riley found that the ability of mercury paints to prevent fouling is related to the leaching rate of mercury (49, 51, 52). He concluded that mercury should be liberated at a rate of about 2 $\mu\text{g}/\text{cm}^2/\text{day}$ in order to prevent fouling. Some results obtained with a group of paints designed by the Naval Research Laboratory for use on aircraft hulls are shown in Table 20.

Barnes (8) found that, when the mercury is released in an inorganic form, a leaching rate of 2 $\mu\text{g}/\text{cm}^2/\text{day}$ is adequate to prevent fouling. In

TABLE 19. Pigment Composition and Leaching Rates of Modifications of Formula 16A, Containing Constant Total Volume of Pigment

Paint No.	Pigment Composition (lb/100 gal)		Copper Leaching Rates, $\mu\text{g}/\text{cm}^2/\text{day}$ After Soaking				
	Cu ₂ O	Metallic brown	1 mo.	2 mos.	3 mos.	4 mos.	6 mos.
1	0	725	0.6 ^f	0.6 ^f	0.3 ^f	0.0 ^f	— ^f
2	50	695	6.9 ^f	1.8 ^f	0.6 ^f	0.3 ^f	1.6 ^f
3	100	664	8.1	— ^f	0.6 ^f	0.3 ^f	1.6 ^f
4 ¹	200	600	13.5	6.0 ^f	0.9 ^f	1.2 ^f	2.3 ^f
5	400	476	24.9	19.5	7.5 ^f	8.4 ^f	2.0 ^f
6	600	351	26.7	17.7	20.1	11.4	— ^f
7	800	226	27.6	23.4	22.8	16.5	25.0 ^f
8	1000	153	28.2	23.7	21.6	17.7	29.3 ^f
9	1160	0	38.4	23.4	24.6	18.3	29.9 ^f

¹ Original formula 16A. Vehicle composition: Rosin, 225; Cresol, 218; Coal tar naphtha, 270 lb/100 gal.

^f Paint fouled when exposed at Miami Beach, Florida.

TABLE 20. Leaching Rates of Various Paints Containing Mercury Compounds as the Toxic

Paint No.	Mercury Compound Used	Mercury Leaching Rates, $\mu\text{g}/\text{cm}^2/\text{day}$ After Soaking					
		1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.
17	HgO, Cu ₂ O	1.1 ^f	2.7 ^f				
82	HgCl	1.8	0.9 ^f	1.0 ^f	2.2 ^f	2.7 ^f	3.4 ^f
85	HgCl	2.0	2.7 ^f	1.9 ^f	2.2 ^f	—	2.1 ^f
93-4	HgO	3.0	1.6	1.0 ^f	1.8 ^f	1.6 ^f	—
87	HgCl	5.5	1.4	1.4	3.1	2.8	3.8
93-3	HgO	5.2	10.7	7.7	12.5	7.2	—
93-9	HgCl ₂	20.0	7.6	3.5	2.3	1.6	—

Fouling resistance unsatisfactory.

some paints, however, the mercury combines to form soaps with the matrix ingredients. These paints are less toxic, and leaching rates of even $6 \mu\text{g}/\text{cm}^2/\text{day}$ were found inadequate to prevent fouling.

It has been claimed that paints which contain both cuprous oxide and a mercury compound are more effective than those which contain either

quoted in the previous section (50) showed that, in solutions, the toxicity of copper and mercury are strictly additive. Thus, a half lethal concentration of copper, combined with a half lethal concentration of mercury, gives a solution which is just lethal. If this same relationship holds true in paints, it would be expected that the leaching rates would be additive proportional to their respective minimal values. There are not sufficient data available to test this hypothesis.

The evidence shows that the successful anti-fouling formulations prevent the attachment or growth of fouling forms because of the liberation of one or more toxic ingredients. Some of the other mechanisms which have been proposed to explain antifouling action may contribute in a secondary way, but no practical means has yet been discovered to prevent fouling without toxic action.

Effects of Slime Films on the Toxicity of Paint

The slime film which forms on a paint surface accumulates large quantities of the toxics dissolved from the paint. The slime may contain as much as a thousand times the toxic concentration found in a saturated sea water solution. The slime film may improve the performance of the paint by keeping a relatively high concentration of toxic at the surface, or by increasing the leaching rate of the paint because of bacterial decomposition of the matrix. It may decrease the effectiveness of the paint if the toxic is present in a combined less poisonous form, or if the maintenance of a high concentration at the surface decreases the rate of dissolution of the toxic from the paint. A brief discussion of the distribution of toxic in the slime, and of the nature of the compounds formed, will aid in interpreting experiments designed to determine its effect on fouling.

The large quantities of copper found in the slime film must be present in a combined form. If a slime film is formed on a nontoxic panel and placed in sea water which contains copper, the copper is rapidly removed from solution and concentrated in

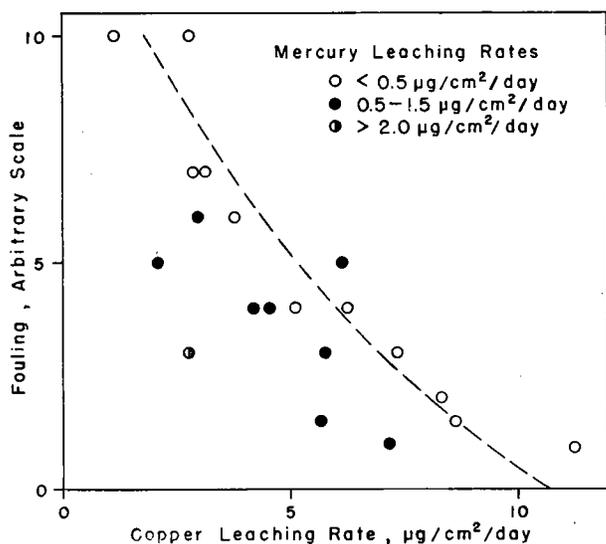


FIGURE 16. Fouling resistance related to the copper leaching rate of paints with various mercury leaching rates. After Harris & Forbes (23).

toxic alone. As yet it has been impossible to determine the way in which the two leaching rates combine in such paints to give a satisfactory anti-fouling performance. It is certain, however, that some cuprous oxide paints, which are borderline in effectiveness can be made more effective by the addition of fairly small quantities of mercury.

Harris and Forbes (23) found that mercury leaching rates of 1-2 micrograms per square centimeter per day impart a considerable degree of antifouling effectiveness to paints having copper leaching rates well below the critical value. Such combined mercury and copper leaching rates, however, were not adequate to prevent fouling, as shown in Figure 16. The toxicity experiments

TABLE 21. Proximate Distribution of Copper in Slime Films Collected from Hot Plastic Paint (142), Coal Tar Rosin Paint (15RC), Wood and Glass Surfaces

Nature of Surface	142	142	15RC	15RC	Wood	Wood	Glass
Copper in Slime mg/gram	8.05	7.75	3.62	1.80	0.133	0.084	1.50
Water Soluble %	6.6	7.2	12.1	11.4	2.4	1.6	26.1
Ether Soluble %	0.3	0.2	0.2	0.2	1.3	0	0.4
Alcohol Soluble %	9.6	6.2	13.6	21.2	21.3	12.9	21.9
Dilute HCl Soluble %	83.5	86.2	74.1	65.0	75.0	85.5	50.9
Residue %	0	0.2	0	2.2	0	0	0.7

the slime. If such a slime film is placed in a container of sea water with a toxic paint surface, no increase in the copper in solution is observed for several days; but the copper content of the slime increases during this time, indicating that the accumulation of toxic by the film is a rapid and active process (64).

The distribution of copper in various fractions of the organic matter in the slime film was studied by Waksman, Ketchum, and Davidson (58). Most of the copper in the slime, 40 to 60 per cent, is dissolved out by sea water saturated with carbon dioxide, and practically all of it is dissolved by a dilute hydrochloric acid solution (58, 60). The distribution of copper in the ether soluble, alcohol soluble, and hydrochloric acid soluble fractions for seven films is shown in Table 21. These results suggest that most of the copper in these films is precipitated as inorganic compounds. A small but appreciable amount is combined with the ether and alcohol soluble organic materials.

These results suggest that the pH will determine the concentration of soluble copper in the slime (cf. Chapter 15). Bray (11, 12), in his studies of the slime film, found values as low as pH 6, and numerous determinations by Whedon (66, 67, 68) gave values from pH 7.5 to pH 8.0. Renn (48) determined that the CO₂ produced by the slime bacteria might decrease the pH by 0.6 units. Such a change would increase the rate of solution of cuprous oxide about fourfold and might, there-

fore, contribute to the effectiveness of the surface.

The amount of slime film which forms on a paint surface, as well as its copper content, is influenced by the composition of the paint. Effective copper paints may inhibit the development of the slime, but the slime which does form contains a high concentration of the toxic. Data are given in Table 22 which show the magnitude and copper content of the slimes which formed on a series of paints containing graded amounts of cuprous oxide. The leaching rates of these paints were given in Table 15. The paints containing the greatest amounts of cuprous oxide formed the least slime, but the concentration of copper in these slimes was much greater than that in the slimes which formed on the less toxic paints. During the first month of immersion at Miami, Florida, the seven paints containing the most copper remained free of fouling. These paints had high leaching rates, and the slime films which formed on them accumulated large amounts of copper from the paint.

Phelps (44) studied the effect of the slime on the attachment of barnacle larvae to a toxic paint. The larvae, settling during each 24 hour period on both a fresh panel and on a panel continuously exposed, were recorded for 20 consecutive days. The total number attached to the continuously exposed panel was never significantly greater than the number which attached to the fresh panel during the previous 24 hours. These results, some of which are given in Table 23, show that, whatever

TABLE 22. Magnitude and Copper Content of the Slimes Formed on Cold Plastic Paints Containing Various Amounts of Cu₂O After Two Weeks' Immersion at Woods Hole, Massachusetts

Paint	Cu ₂ O Paint %	Dry Wt. of Slime μg/cm ²	Copper in Slime μg/cm ²	Copper in Slime %	Fouling Resistance Miami 1 Month
C-1	0.4	2220	0.71	0.032	73
C-2	1.1	1710	0.97	0.057	60
C-3	1.8	952	0.71	0.074	64
C-4	3.6	770	0.87	0.113	89
C-5	7.1	339	0.70	0.206	100
C-6	12.1	319	1.07	0.336	100
C-7	16.7	135	1.06	0.785	100
C-8	19.7	187	1.24	0.665	100
C-9	23.9	157	1.02	0.644	100
C-10	30.7	216	2.40	1.11	100
C-11	39.0	122	0.98	0.80	100

: Expressed as % of the total dry weight of the slime film.

the explanation may be, the continuously exposed panels were more resistant than fresh panels to the attachment of cyprids. Although the concentration of toxic in the slime film was not measured in Phelps' experiments, copper concentrations of 0.35 to 0.86 mg. of copper per ml., and 0.025 to 0.115 mg. of mercury per ml. of the slime on this paint were found in other exposures at Beaufort (28).

Experiments on the attachment and growth of *Bugula* larvae have confirmed and extended these general observations (39, 40, 66, 68). These tests were made on painted panels which were seasoned in sea water in the laboratory for various periods to permit the development of slimes. The panels were then exposed to larvae of *Bugula* after the removal of the slime film from some of the panels. From these experiments the authors concluded that the influence of the slime film on toxic paints was slight, since very few larvae were able to attach to slimed or cleaned surfaces. In most cases the presence of the slime film either made no difference or else increased slightly the attachment of the *Bugula* larvae to the effective paints. No growth of *Bugula* occurred on any of the effective surfaces, regardless of the presence or absence of the slime film. The copper content of the slime films was

TABLE 23. Comparison of Attachment of Barnacles to Fresh and to Slimed Antifouling Paint Panels (44)

Paint 15RC Exposed at Beaufort, N. C.

Exposure Days	Fresh Panels No/cm ² /day	Total No/cm ²	Slimed Panels Total No/cm ²
2	2.24		2.24
3	1.26	3.50	2.52
4	0.86	4.36	1.80
5	1.81	6.17	1.13
6	2.75	8.92	0.89
7	2.48	11.40	0.77
8	2.41	13.81	0.68
10	1.94	15.75	0.75
15	0.77	16.52	0.45
19	0.97	17.49	0.63

of the slime on the paint surface may diminish the leaching rate from the paint. Experiments by Whedon (69) and Riley (51) indicate that the slime does, in most cases, decrease the amount of toxic escaping to the surrounding sea water. These effects of the slime film are discussed in Chapter 16.

The extensive investigations of slime films have not resulted in any significant correlation between the amount of slime or its physical properties and the ability of the surface to prevent fouling, except insofar as the most effective toxic paints both decrease the amount of slime and prevent the attachment of fouling. The most important property of

TABLE 24. Attachment and Growth of *Bugula* Larvae to Paints Containing Graded Amounts of Cuprous Oxide After Exposure for Four Weeks in Sea Water to Permit Development of the Slime

Paint	Cu ₂ O in Paint %	% Attachment		Growth (mm.)		Copper in Slime ¹
		Slime Present	Slime Removed	Slime Present	Slime Removed	
—	0	76	39	1.4	1.2	—
5C	7.1	62	81	0.9	0.7	1.44
7C	16.7	13	17	0.2	0.2	6.39
9C	23.9	2	8	0.2	0.2	6.40
10C	30.7	3	4	0.2	0.2	6.73

¹ Expressed as % of organic matter.

also determined, and large attachments and good growth were observed only on the paints which produced slimes containing little copper. When the copper content of the slime was above 6 per cent, very little attachment was observed. An example of the data on which these conclusions were based is given in Table 24. This experiment used paints similar to those described in Table 22.

The matrix ingredients of the paints studied may also affect the nature of the slime film which forms on the surface. Renn (48) and Darsie (15) have shown that the bacteria of the slime can decompose rosin and other related paint ingredients. The utilization of a paint component by the slime bacteria may result in more rapid breakdown of the paint film, and can thus result in the more rapid release of the toxic of the paint. The presence

of the slime appears to be its content of the toxics which it derives from the paint film, and the effect the slime may have on the leaching rate of the paint.

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