Degradation of 1-Naphthol in Sea Water

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The fate of 1-naphthol-1-^{14}C was studied in a simulated estuarine environment since 1-naphthol is formed by the hydrolysis of carbaryl used for the control of ghost shrimp in oyster beds. 1-Naphthol is unstable in the alkaline environment of sea water. Light and microorganisms enhance the degradation of 1-naphthol to CO_2 and numerous other products. 1-Naphthol is relatively stable in an oxygen-free aqueous solution and in the presence of certain cations. A major portion of the 1-naphthol formed a precipitate in sea water having a molecular weight of 454 a.m.u. The precipitate contained a stable free radical and was toxic to certain estuarine species. The persistence of the precipitate may explain an observed lack of recolonization of carbaryl treated areas.

Use of carbaryl, 1-naphthol N-methylcarbamate, for predator control in oyster beds by Loosanoff et al. (1960), Lindsay (1961), and Haydock (1964), has stimulated experimental application in Yaquina Bay, Ore., by the Oregon State University Marine Science Center. As with any toxic substance applied to a biological system, the products produced by the system may be of major importance owing to their toxic effects.

Carbaryl has been shown to be readily hydrolyzed to 1-naphthol in sea water (Karinen et al., 1967); however, 1-naphthol was rapidly converted to other products. 1-Naphthol is more toxic than carbaryl to young clams at a concentration of 6.4 p.p.m., and twice as toxic to fish at 1.3 p.p.m. (Stewart et al., 1967); thus the effects of its presence are biologically important.

Crosby et al. (1965) found six cholinesterase inhibiting spots using TLC-cholinesterase techniques after irradiation of carbaryl in organic solutions. Kawasaki (1965) has followed the oxidation of naphthols in 5% NaOH solutions. He obtained high yields of phthalic acid and small amounts of 3,8-pyrenequinone, 9,10-dioxynaphthacenequinone, and hydroxybenzoquinones from 1-naphthol.

In this study, the fate of 1-naphthol-1-^{14}C in a simulated marine estuarine environment was investigated to determine if any potential hazard exists in the use of carbaryl in oyster beds. The effects of oxygen, light, microorganisms, pH, and metallic salts on the stability of 1-naphthol were also investigated.

EXPERIMENTAL

Materials and Methods. 1-Naphthol-1-^{14}C was obtained from Amersham/Searle Corp. with a purity of 99+%. The purity of both labeled and nonlabeled 1-naphthol was determined by thin-layer chromatography on silica gel G using 4:1 ether:hexane as the mobile solvent. Sea water obtained from Yaquina Bay, Ore., was filtered before use, and mud was taken from flats similar to those used for oyster beds. 1-Naphthol was determined by the colorimetric method reported by Karinen et al. (1967).

1-Naphthol in Sea Water. Seven sea water aquaria were set up using 2-liter reagent bottles for tanks. A coarse glass frit for aeration of the water and a gas exit tube were fitted into a two-hole rubber stopper used to seal the tanks. A gas scrubber containing 2N NaOH was connected to the exit tube to trap $^{14}CO_2$ evolving from the tanks. Atmospheric pressure was maintained in the tanks by attaching a vacuum pump to the exit side of the gas trap and an air pump to the aeration frit, with valves on both the vacuum and air pump lines to adjust the pressure in the tanks to atmospheric pressure.

Experimental conditions for the tanks are shown in Table I. In the sterile system, the tanks, sea water, and aeration apparatus inside the tanks were autoclaved intact for 1 hour at 120° C and 248 p.s.i. The light tanks were exposed to two 8-foot Gro-Lux fluorescent lights (Sylvania Electric Products Inc., Danvers, Mass.) for 10 hours daily to simulate daylight conditions. The dark tanks were covered with aluminum foil to maintain continuous darkness. All tanks were kept at 16 ± 1° C.

1-Naphthol-1-^{14}C (28.6 mg.) with a specific activity of 0.047 mCi. per mM. was dissolved in 5 ml. of 2-methoxyethanol and swirled into each tank under the surface of the water. A fine precipitate formed, but readily dissolved. Five milliliters of 2-methoxyethanol were added to each control tank.

Samples were taken after 2 hours, and again at 2, 5, 9, 13, 16, and 24 days. One-milliliter samples were analyzed for 1-naphthol colorimetrically, and 0.2-ml. samples were added to 10 ml. of 2:1 toluene:2-methoxyethanol containing 5.5 grams per liter 2,5-diphenyloxazole (PPO) for liquid scintillation counting. At each time period 0.2 ml. of the CO_2 scrubber solution was added to 10 ml. of counting solution for the determination of $^{14}CO_2$. After 24 days, the water was filtered through a Mf-Ha Millipore filter (0.45 micron mean pore size) to remove a reddish-blue precipitate which had formed in all of the systems.

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Table I. Experimental Conditions and Fortification Level of Aquaria

Tank Number		Fortification	
Sea water only[a]	Sea water and mud	P.p.m.	μCi/mM.
1 l,s		Control	...
2 d,u		Control	...
3 l,u		Control	...
4 d,s		14.3	0.047
5 l,s		14.3	0.047
6 d,u		14.3	0.047
7 l,u		14.3	0.047
	8 l,s	Control	...
	9 d,u	Control	...
	10 l,u	Control	...
	11 d,s	15.0	0.063
	12 l,s	15.0	0.063
	13 d,u	15.0	0.063
	14 l,u	15.0	0.063

[a] l = light, d = dark, s = sterile, u = unsterile.
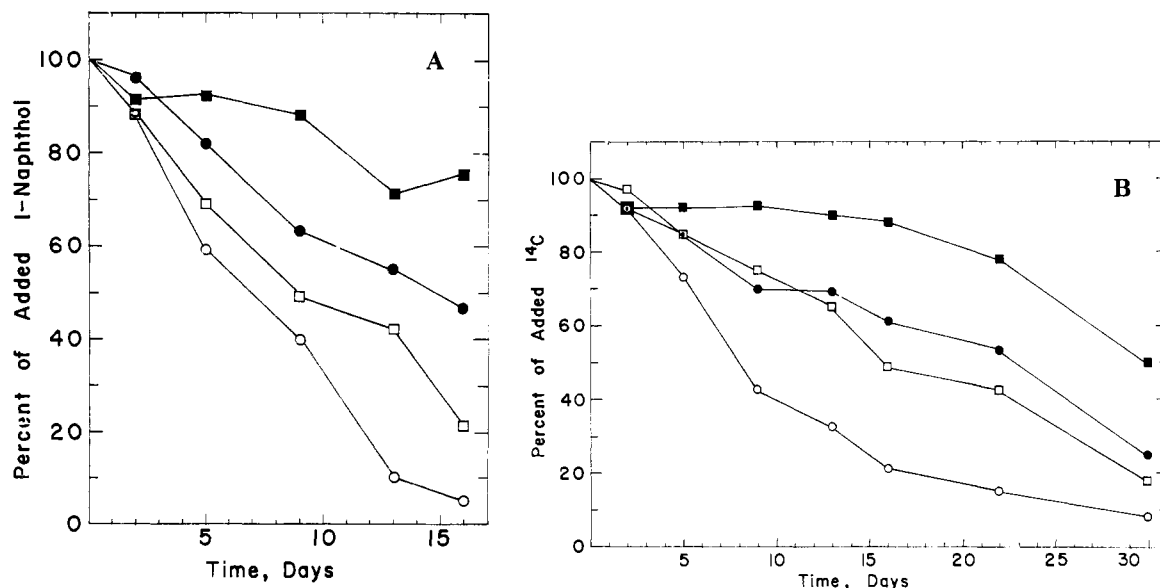
Figure 1. Effect of light and microorganisms on stability of 1-naphthol in sea water

a. Loss of 1-naphthol   ○ Light, unsterile   □ Light, sterile
b. Loss of total ^{14}C ● Dark, unsterile   ■ Dark, sterile

1-Naphthol in Sea Water and Mud. Seven sea water aquaria were set up as described above and 1½ inches of mud (approx. 500 g. dry weight) was placed in the tanks with 1.5 liters of sea water (Table I). Samples were taken as before. Upon termination at 33 days, the unsterile tanks which had produced $^{14}CO_2$ were aerated with shaking to release any mechanically trapped gases in the mud. At the end of an hour the aeration tube was clamped and the tank evacuated to 10 mm. through the gas scrubber to further trap any $^{14}CO_2$.

After purging, the water was filtered through a Whatman No. 1 filter to separate the mud from the water. The water was extracted with an equal volume of dichloromethane (DCM) and aliquots of 0.2 ml. were taken to determine the amount of radioactivity in the water and DCM. The mud was extracted 8 times with 250 ml. of acetone, and filtered to dryness each time. A 0.2-ml. aliquot of the combined acetone extracts was counted. A 10-gram portion of dried mud was then heated with 50 ml. of dimethyl sulfoxide (DMSO) for 24 hours on the steam bath, and the DMSO withdrawn with a pipet. Since additional activity was extracted from the soil, a total of four extractions were made in this manner.

Effects of Oxygen, pH, and Cations. To determine the role of oxygen on the stability of 1-naphthol, 1 liter of a NaH_2PO_4-NaOH buffer solution (pH 7.8) was placed in a 2-liter reagent bottle. The bottle was stoppered with a two-hole rubber stopper containing a coarse glass frit with an exit tube. The apparatus and water were sterilized and purged with 100 ml. per min. nitrogen gas for 5 days. The nitrogen gas was passed through copper wool in a 850° C combustion furnace to remove traces of oxygen. After purging, the frit was sealed and a sterile septum placed over the exit tube. The system was cooled to 16° C and fortified with 1-naphthol. Samples were removed weekly with a sterile syringe through the septum. After 30 days, air, drawn through a cotton filter to remove the microorganisms, was passed through the solution and samples were again taken.

To test the dependence of the stability of 1-naphthol on pH and the presence of cations, aquaria containing 500 ml. of distilled water were adjusted to pH 4.4, 5.5, 6.3, 7.3, 8.0, and

8.5 with 5 ml. of 0.1M Trizma buffer (Sigma Chemical Co., St. Louis, Mo.). Each aquarium was fortified at 0.128 mM (21.2 p.p.m.) of 1-naphthol. The aquaria were maintained at 16 ± 1° C and continuously exposed to Gro-Lux fluorescent lights. Magnesium chloride (0.013M), sodium chloride (0.145M), and calcium chloride (0.004M) were added separately to three additional aquaria which were buffered at the pH of sea water (pH 8.2).

Thin-Layer Chromatography. Compounds extracted from the sea water and mud were separated on 250-micron 20 × 20 cm² silica gel G (Warner-Chilcott Laboratories) plates. Radioactive compounds were detected by "no-screen" x-ray film (Eastman Kodak Co.). Naphthol compounds which were not substituted at the 4 position were detected with a 5% NaOH spray followed by a p-nitrobenzenediazonium fluoborate saturated MeOH spray (Finocchiaro and Benson, 1965), or a methanol spray saturated with tetraazotized O-dianisidine. To locate other compounds formed, 5% rhodamine 6G in acetone was sprayed on the plates and visualized with short wave ultraviolet light. The TLC plates were also sprayed with 5% sodium hydroxide and observed under long and short wave ultraviolet light to visualize any dihydroxynaphthalenes.

Identification of a Reddish-Blue Precipitate. A reddish-blue precipitate which formed in all of the 1-naphthol fortified aquaria was filtered from the sea water with Whatman No. 42 paper, washed with distilled water, and vacuum dried. Part of the precipitate was dissolved in acetone and spotted on a TLC plate. Another portion was dissolved in DMSO for spectroscopic measurement. N.M.R. and e.p.r. spectra were obtained on Varian A-60 and E-3 spectrometers. Mass spectra were obtained on an Atlas Model CH-7. A dry portion of the precipitate was inserted into the ion source by a direct access probe. The sample was kept at ambient temperature for a few minutes to allow the ion source pressure to return to background. The probe was then temperature programmed at 10° C/minute until the pressure began to rise. The temperature was then kept constant until the pressure returned to near background. The probe temperature pro-

Table II. Distribution of Radioactivity Used in Both Radiotracer Experiments

Distribution of Radioactivity

Tank Number[a]	Exp. 1 1-Naphthol + Sea Water in % of Amount added				Exp. 2 1-Naphthol + Sea Water + Mud in % of Amount Added			
	7 l,u	6 d,u	5 l,s	4 d,s	14 l,u	13 d,u	12 l,s	11 d,s
CO_2 evolved	14.25	1.86	0.01	0	16.1	29.4	...	...
Total ^{14}C in water	8.4	25.1	16.3	49.0	2.2	1.8	24.4	31.9
^{14}C extractable from H_2O (DCM)	2.0	15.6	1.6	37.8	0.22	0.29	9.00	15.50
Radioactivity precipitated from sea water	65.3	44.7	83.5	52.0				
Acetone extractables from mud					14.0	9.4	21.9	31.5
DMSO					42.2	34.8	23.9	15.8
Total extractable from mud					56.2	44.2	45.8	47.3
Total Recovery	87.7	71.8	99.6	101.0	75.5	75.4	69.4	79.2

[a] l = light, d = dark, s = sterile, u = unsterile.

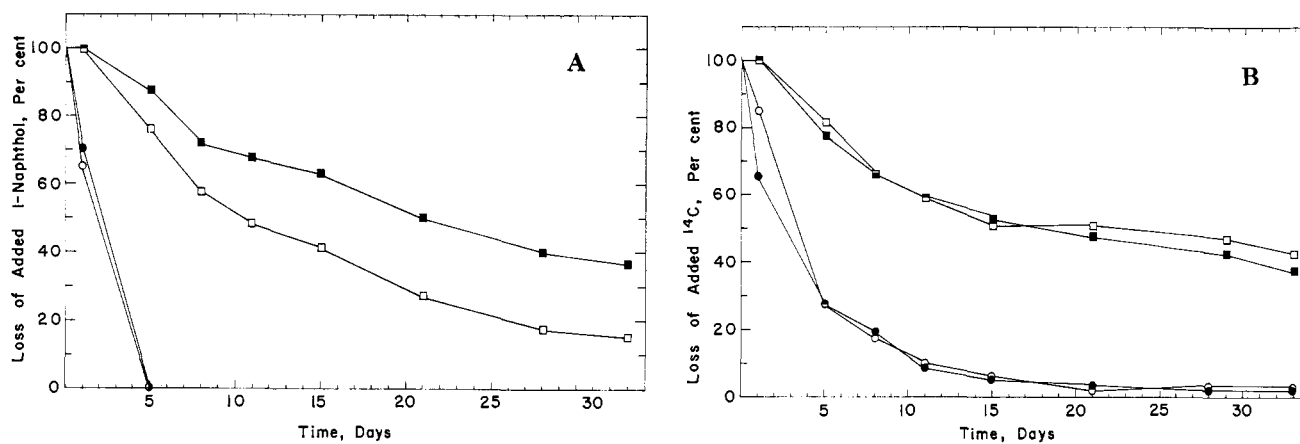
Figure 2. Effect of light and microorganisms on stability of 1-naphthol in sea water with mud present

a. Loss of 1-naphthol	○ Light, unsterile	□ Light, sterile
b. Loss of total ^{14}C	● Dark, unsterile	■ Dark, sterile

gram was continued again until another pressure increase was observed. The total ion monitor recorded the vaporization of the precipitate with temperature.

RESULTS AND DISCUSSION

1-Naphthol in Sea Water. The stability of 1-naphthol in sea water is affected considerably by light and microbial action, as shown by the comparison of light and dark, and sterile and unsterile tanks (Figure 1a). 1-Naphthol is more stable in dark systems than in light systems; in either case, the presence of microorganisms will accelerate the loss of 1-naphthol from the water. The loss of radioactivity in sea water was similar to the loss of 1-naphthol-1-^{14}C, but at a slower rate (Figure 1b).

The chemical compounds obtained from the degradation of 1-naphthol in sea water were different in the presence and absence of light. A comparison of the amount of dichloromethane (DCM) extractable radioactivity from the water of the light and dark tanks shows that the metabolites formed in the dark tanks are mostly soluble in DCM while those in the light tanks show slight DCM solubility.

Since $^{14}CO_2$ was found in only the two unsterile tanks, it may be concluded that the $^{14}CO_2$ was produced by microorganisms. Although both unsterile tanks yielded $^{14}CO_2$, the tanks exposed to light produced approximately 8 times as much $^{14}CO_2$ as the dark tanks (Table II), indicating that the presence of light enhances $^{14}CO_2$ production by the microorganisms.

A precipitate which formed in all of the tanks was noticeable by the third day in the light exposed tanks. The rate of formation of the precipitate concurred with that of the loss of 1-naphthol. By the 16th day, the red color became so intense that it severely interfered with the colorimetric determination. Filtering removed red particulate matter, but the solution remained a red color.

The recovery of the added radioactivity in the light and dark unsterile tanks was 87.5% and 71.7%, respectively (Table II, tanks 6 and 7). Approximately all of the radioactivity was accounted for in the sterile tanks (tanks 4 and 5). The loss of radioactivity in the unsterile tanks may be the result of a gas, such as methane, being evolved and not trapped by the NaOH solution.

1-Naphthol in Sea Water and Mud. Five to ten minutes after fortification of the tanks containing mud, juvenile ghost shrimp appeared in distress. The worms present did not seem to be affected since they continued to burrow in the mud throughout the experiment. Gas bubbles appeared on the
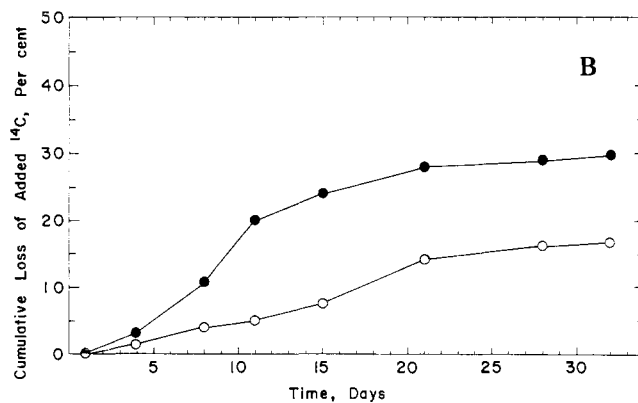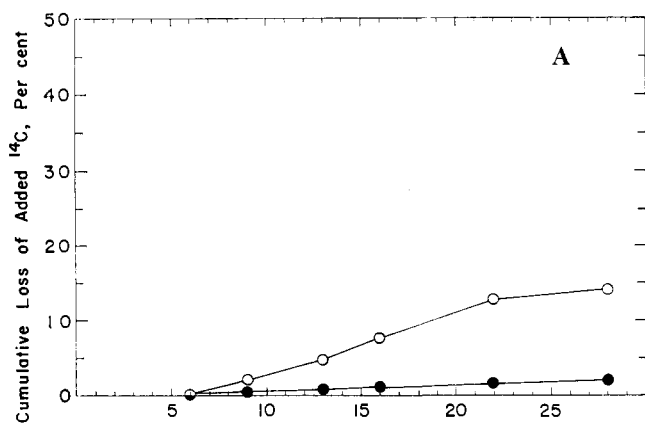
Figure 3. Production of $^{14}CO_2$ from 1-naphthol-1-^{14}C under light and dark conditions

a.	In sea water	○ Light
b.	In sea water over estuarine mud	● Dark

surface of the mud in about 4 days; but were not so numerous by the 15th day. The sterile tanks showed no bubbles on the mud surface.

The loss of 1-naphthol from sea water is much faster in the presence of mud (Figures 1*a* and 2*a*). This loss is the result of adsorption and the increased populations of microorganisms in the mud. The total radioactivity declines in the sea water of both the dark and light sterile tanks at nearly the same rate (Figure 2*b*). The same is true of the unsterile tanks. As with 1-naphthol, adsorption by the mud seems to be the major factor involved.

No $^{14}CO_2$ was detected in the sterile systems as in the sea water only experiment. A comparison of the amount of $^{14}CO_2$ evolved from the unsterile tanks of the sea water only systems with those of the system with mud present shows a reversal in the amount obtained from each tank (Figures 3*a* and 3*b*). The fact that there is mud present containing different kinds of microorganisms may be the only explanation for this.

Exhaustive extraction of the mud with acetone yielded approximately one third of the radioactivity in the mud. Further extraction with DMSO released the remaining two thirds from the mud. These highly polar compounds are not readily extractable from the DMSO, and have not been separated for identification or further characterization.

The overall recovery of radioactivity from this system was about 75% for each of the tanks with most of the radioactivity being found in the mud. Although the mud was extracted four times with DMSO, it is probable that all of the radioactivity was not removed. The last DMSO extraction contained less than 2% of the radioactivity that had initially been added to the system.

Effects of Oxygen, pH, and Cations. In a sterile, light-exposed, oxygen-free solution, the concentration of 1-naphthol decreased 0.3% per day for 30 days. After the addition of oxygen, the concentration decreased 1.6% per day for the next 40 days. Thus 1-naphthol is relatively stable in the absence of oxygen and the presence of light. Its degradation in water may be attributed more to photo-oxidation than photo-decomposition.

Trizma maleate and Trizma base were used to buffer the aquaria involved in the pH and cation study to avoid the presence of cations. The stability of 1-naphthol at different pH values is given in Figure 4. 1-Naphthol has an optimum stability at a pH of 6.3; and is unstable at 8.2, the pH of sea water. The addition of cations similar to those found in sea water and at a similar concentration did not affect the rate of
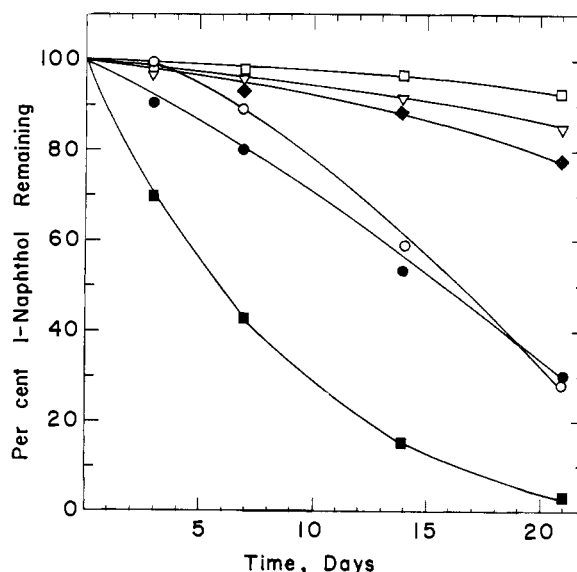

Figure 4. Effect of pH on stability of 1-naphthol

○	pH 4.4	◆	pH 7.3
▽	pH 5.5	●	pH 8.0
□	pH 6.3	■	pH 8.5

degradation of 1-naphthol. Thus the degradation of 1-naphthol in sea water is principally affected by the presence of oxygen, light, microorganisms, and the pH of the solution.

TLC. The number and types of products formed by the degradation of 1-naphthol were examined by TLC. The precipitate from the sea water only tanks, the acetone extracts of the mud, and the DCM extracts of the water from both systems were each compared by TLC. The combined results from the TLC plates are shown in Table III for a 4:1 ether:hexane system. The major portion of the radioactivity was found at the origin. A large spot at a R_f of 0.06–0.08 was found in the unsterile systems. Cochromatography of dihydroxynaphthalenes, 1,4- and 1,2-naphthoquinones, 5-hydroxy- and 2-hydroxy-1,4-naphthoquinones with extracts of compounds having similar R_f's showed that less than 1% of these compounds may have been present.

Identification of Reddish-Blue Precipitate. A reddish-blue precipitate (I) which forms in all of the 1-naphthol fortified aquaria was not resolved by the various TLC systems tried. Infrared spectroscopy of a DMSO solution of the precipitate and KBr pellets showed bands of particular interest at 3600, 3220, and 770 cm^{-1}. The out-of-plane C—H bend for 4

| | | Sea Water Only | | | | Sea Water + Mud | | | |
| | | Unsterile | | Sterile | | Unsterile | | Sterile | |
Metabolite Areas		7 light	6 dark	5 light	4 dark	14 light	13 dark	12 light	11 dark
XII	(0.87)					T			
XI	(0.79–0.76) 1-naphthol	++	++	+	+	+	+	++	++++
X	(0.71–0.69)			+	+				
IX	(0.63–0.62)	T	T		T			T	
VIII	(0.55–0.51)	T	T		+				
VII	(0.44)		T						
VI	(0.35–0.32)				T	T	T	T	T
V	(0.28–0.26)	++		+		T	T		
IV	(0.21–0.16)		T						
III	(0.08–0.06)	+++		+		+++	+++		
II	(0.03–0.02)	+					T	T	
I	(0.0)	++++	++++	++++	++++	++++	++++	+++	+++

T = Trace < 10% Adsorbent: Silica Gel G
+ = visible (easily) Layer Thickness: 250 microns
++ = small area Solvent System: Ether:Hexane (4:1)
+++ = medium area
++++ = large area

adjacent hydrogens at 770 cm^{-1} and the absence of a three adjacent C—H bend band (750–810 cm^{-1}) indicates one ring of 1-naphthol is intact while the other ring has undergone substitution. The 3600 cm^{-1} band may be due to hydrogen bonded OH (Wang et al., 1959). The n.m.r. spectra showed broad peaks at 3.6 and 7.3 p.p.m. using tetramethyl silane as an external reference. The presence of a broad peak in the n.m.r. spectra could be due to either an exchange of protons among different species or the presence of a free radical in solution. The presence of a free radical in the solution can cause significant changes in the line width and a chemical shift of the resonance peak as a result of hyperfine interaction between the free electron and the proton. The e.p.r. spectra of a DMSO solution showed a sharp resonance peak around the g-value for a free electron (2.0).

A molecular weight of 450 was obtained by the Rast method which agrees with mass spectroscopy data. Four peaks were obtained on the total ion monitor of the mass spectrometer at 70, 250, 360, and 500° C. The spectra at 70° C showed a compound with a molecular weight of 279, but no assignments could be made. The spectra at 250° C was completely assigned to 1,4-naphthoquinone (Bowie et al., 1965). The spectra at 360° showed a compound with a molecular weight of 454 and a base peak of 298. Peaks at a m/e of 174 and lower showed the presence of 2- (or 3)-hydroxy-1,4-naphthoquinone (m/e of 174, 146, and 105); 1,4-naphthoquinone (m/e of 160, 158, 131, 130, 104, and 102); and 1-naphthol (m/e of 144, 116, and 115). No assignments were made to peaks above 174. At 500° C, a compound with a molecular weight of 454 was again obtained but the fractionation pattern was different from the spectra at 360° C. The base peak was 284. At the lower end of the spectra 1-naphthol and 1,4-naphthoquinone were clearly present, but identification of the intact compound was not made. The precipitate must thermally fractionate at the different temperatures since 1-naphthol and 1,4-naphthoquinone have sufficient vapor pressure at am-

bient temperature to be removed from the samples.

Toxicity of the Precipitate. The toxicities of sea water solutions containing the precipitate found in the 1-naphthol experiments were checked using bay mussel embryos and compared with similar tests using 1-naphthol. The precipitate was found to be two thirds as toxic as 1-napthol. This may be a result of the presence of a stable free radical. The precipitate also appeared to inhibit cholinesterase. These findings tend to support observations of the treated mud flats which failed to recolonize 18 months after treatment with carbaryl. Since this material is nearly as toxic as 1-naphthol to certain organisms, it should be considered in future studies.

ACKNOWLEDGMENT

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