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SURFACE ACTIVE HIGHER ALKYL TRIMETHYL AMMONIUM
CHLORIDE OIL BIOCIDES OF PREFERENTIAL WATER SOLUBILITY (1)

By

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INTRODUCTION

Most known fuel oil additives such as microbiocides are preferably soluble in oil rather than in water. Of course, for compounds to be added to oil a certain degree of oil solubility is usually essential. However, in the past most oil additive research centered on compounds having preferential solubility in oil. In the area of oil biocides, the importance of preferable solubility in water was recognized but it was felt that "few treating compounds can be added to a hydrocarbon system which will then migrate from the hydrocarbon to the water phase" (2).

The continuing need for oil microbiocides is connected with the presence of water bottoms in refinery and home heating oil tanks. The proliferation in water of fungi and bacteria at the oil interface, increases sludge formation and, consequently, burner filter plugging.

The new concept, in our search for surface active quaternary ammonium salt microbial inhibitors centered on compounds having moderately preferential solubility in water. The basis of our new concept is that microbial attack occurs at the oil-water interface of a system which mostly consists of oil. Consequently, a higher concentration of the inhibitor will be in the region of the interface if the compound is preferably water soluble. On the other hand, preference for water solubility should be moderate since, if most of the oil additive were to concentrate in the water bottoms, protection would be lost due to leaching on repeated transfer.

As potentially attractive novel oil additives, according to the above concept, surface active higher monoalkyl trimethyl ammonium chlorides were evaluated. The antibacterial effectiveness was tested in special laboratory tests, including simulated tank bottom tests.

REVIEW OF RELATED WORK

Higher alkyl trimethyl ammonium salts, especially the chlorides, are known surfactants with high microbiocidal activity. Their synthesis, properties and applications are described in considerable detail in a monograph, entitled "Cationic Surfactants", edited by E. Jungermann (3). Their solubility characteristics in water and other polar solvents were studied by Reck and co-workers (4). However, to date, such salts were considered as useful components mainly in systems containing major amounts of water, e.g., as germicides (3,5). In contrast, in the present work, the microbiocidal properties of such salts were studied in water-in-oil systems consisting of major amounts of various fuels and crude oil.

Unlike higher monoalkyl trimethyl ammonium salts, the higher dialkyl dimethyl ammonium salts are known microbiocides. Several patents assigned to the Exxon Research and Engineering Co. and Gulf Research and Development Co. describe hydrocarbon oils which contain higher dialkyl dimethyl ammonium chlorides and other preferentially oil soluble quaternary salts (6-10). These patents invite experimentation with such salts in general. However, on the whole the prior work suggests that in oil applications the higher dialkyl rather than monoalkyl compounds are useful.

The only successful use of preferably water soluble tetraalkyl ammonium salts was reported in patents in the case of benzyl ammonium chlorides. These compounds were first claimed as fuel biocides by Summers of Gulf in 1961 (11). He showed that the compounds were effective inhibitors of heating oil gels of microbial origin. After our work (1) was completed, a German patent of Japanese researchers was published (12) which also describes the use as microbiocides of several quaternary benzyl ammonium chlorides of preferred water solubility. In this case, the compounds were applied for the treatment of the water bottoms of petroleum product storage tanks. Comparative laboratory experiments were carried out using fungi isolated from jet fuel (Table I).

TABLE I

RECENT JAPANESE DATA ON THE EFFECT OF WATER SOLUBLE QUATERNARY AMMONIUM CHLORIDES ON FUNGI ISOLATED FROM JET FUEL

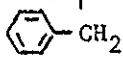

CATION OF CHLORIDE SALT (1 ppm)	CONTACT TIME, Hr.	SURVIVAL, %		
		Clado-sporium	Peni-cillium	Mycelia sterilia
$C_{16}H_{33}N^+Me_2$ 	7	0	0	0
$C_{12}H_{25}$  $CH_2N^+Me_3$	24	0	20	20

TABLE II

DISTRIBUTION OF VARIOUS TYPES OF ORGANO-NITROGEN ADDITIVES BETWEEN HEATING OIL AND WATER

ADDITIVE	NITROGEN, ppm		NITROGEN %, FOUND IN WATER
	ADDED TO OIL	FOUND IN WATER	
None	0	< 1	-
$[CocoN^+Me_3]Cl^-$	268	244	91
$[(Coco)_2N^+Me_2]Cl^-$	252	37	15
$[R^aCH(Me)N^+Me_3]Cl^-$	212	210	99
Grotan ^b	1495	1100	74

^a $R = C_nH_{2n+1}$; $n = 11-20$.

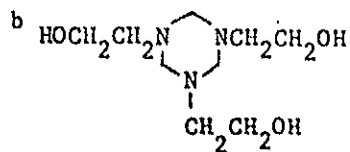


TABLE III

COMPOSITION OF P-1 BASAL SALT MEDIUM

COMPONENTS	GRAMS	SALT "B"	
		SALT	g/l (Water)
$(\text{NH}_4)_3\text{PO}_4$	10	MgSO_4	20
K_3PO_4	5	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	2
Na_2SO_4	0.5	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	1.6
Tap Water (ml)	1000	NaCl	2
"Salt B" (ml)	10		

TABLE IV

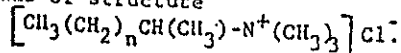
EFFECT OF HIGHER 1-ALKYL AND 2-ALKYL TRIMETHYL AMMONIUM CHLORIDES ON GRAM NEGATIVE BACTERIA UTILIZING DIESEL FUEL

Group	No.	R- Groups of $[\text{R-N}^+(\text{CH}_3)_3] \text{Cl}^-$ Salt	Growth (+) and No Growth (-) at Various Concentra- tions of Salt (ppm) After 24 and 48 Hours					
			(20)		(100)		(500)	
			24	48	24	48	24	48
I	1	$\text{CH}_3(\text{CH}_2)_{11}-$	+	+	+	+	+	+
	2	$\text{CH}_3(\text{CH}_2)_{13}-$	+	+	-	+	-	-
II	3	(Coco- ^a)	-	-	-	-	-	-
	4	(Soya- ^b)	-	+	-	+	-	+
III	5	$\text{CH}_3(\text{CH}_2)_{9-13}-\text{CH}(\text{CH}_3)-$ ^{c,d}	-	-	-	-	-	-
	6	$\text{CH}_3(\text{CH}_2)_{13-18}-\text{CH}(\text{CH}_3)-$ ^{c,d}	+	+	+	+	+	+

^a Monococo trimethyl ammonium chloride as described in Table IV.

^b Soya trimethyl ammonium chloride having primarily n-alkenyl groups of the following composition: 90% C₁₈; 10% C₁₆.

^c A mixture of homologous compounds of structure



^d The composition of the 2-alkyl groups is described in Table V.

TABLE V

EFFECT OF N-DODECYL TRIMETHYL AMMONIUM CHLORIDE
ON GRAM NEGATIVE BACTERIA UTILIZING JET FUEL

Bacterial Strain No.	Growth (+) and No Growth (-) of Bacteria in the Presence of Test Compound				
	Concentration of $[\text{CH}_3(\text{CH}_2)_{11}\text{N}^+(\text{CH}_3)_3]\text{Cl}^-$				
	<u>0 (Control)</u>	<u>10</u>	<u>50</u>	<u>100</u>	<u>500</u>
2-6	+	+	+	+	-
7	+	+	-	-	-

TABLE VI

EFFECT OF PREFERRED WATER VERSUS OIL SOLUBILITY ON
OIL BACTERICIDAL ACTIVITY AS REFLECTED BY
LABORATORY TESTS WITH EXCESS HEATING OIL

Coco- Ammonium Chloride in System, mg	Log Reduction of Bacterial Counts After 7 Days			
	$[\text{CocoN}^+\text{Me}_3]\text{Cl}^-$		$[(\text{Coco})_2\text{N}^+\text{Me}_2]\text{Cl}^-$	
	100 ml Oil	1 ml Oil	100 ml Oil	1 ml Oil
10.00	8	4	4	4
5.00	8	4	4	4
2.50	6	4	3	4
1.20	6	4	1	4
0.60	4	4	0	4
0.30	3	4	0	4
0.15	4	4	0	3
0.07	1	2	0	1
0.04	1	0	0	0
0.02	0	0	0	0

TABLE VII

QUATERNARY AMMONIUM CHLORIDES AS OIL FIELD
BACTERICIDES AGAINST AEROBIC BACTERIA

Structure of Test Compound	Observation Period, Days	Growth (+) or No Growth (-) in the Presence of Various Concentrations (ppm) of Test Compound				
		0	12.5	25	50	100
$[C_{14}H_{29}N^+(CH_3)_3]Cl^-$	1	+	+	-	-	-
	7	+	+	+	-	-
	14	+	+	+	-	-
$[(Coco)_2N^+(CH_3)_2]Cl^-$	1	+	+	+	-	-
	7	+	+	+	+	-
	14	+	+	+	+	-

TABLE VIII

EFFECTIVENESS OF HIGHER MONOALKYL TRIMETHYL
AMMONIUM CHLORIDES AS OIL FIELD
BACTERICIDES AGAINST ANAEROBIC BACTERIA

Quaternary Ammonium Chloride Tested	Observation Period, Days	Growth (+) and No Growth (-) in the Presence of Various Concentrations (ppm) of Test Compounds			
		0	12.5	25	50
$[C_{12}H_{25}N^+(CH_3)_3]Cl^-$	4	+	-	-	-
	7	+	+	-	-
	14	+	+	-	-
$[C_{14}H_{29}N^+(CH_3)_3]Cl^-$	4	+	-	-	-
	7	+	-	-	-
	14	+	-	-	-
$[(Coco)_2N^+(CH_3)_2]Cl^-$ ^a (Control)	4	+	-	-	-
	7	+	+	-	-
	14	+	+	+	-

These and field experiments showed that the compounds were effective fuel fungicides.

The most up-to-date discussion of microbiocides for petroleum products is found in the proceedings of a 1972 London conference of the Institute of Petroleum (13). Unfortunately, none of the papers of this conference considered the quaternary ammonium salt biocides.

RESULTS AND DISCUSSION

In the present work, the microbiocidal effects of readily available surface active quaternary higher monoalkyl and dialkyl ammonium chlorides were studied. A higher trialkyl derivative, tri-n-octyl methyl ammonium chloride was also used for comparison. Most of the studies of course centered on the higher monoalkyl trimethyl ammonium chlorides.

As a higher monoalkyl trimethyl ammonium chloride, the readily available monococo compound will be discussed first. This will be followed by the results on the effect of such salts on bacteria metabolizing middle distillate and crude oils. The oil microbiocidal data will be discussed with emphasis on the effect of the oil versus water solubility of quaternary salts.

In the following, the oil versus water distribution of quaternary higher alkyl ammonium salts will be discussed first. This will be followed by the results on the effect of such salts on bacteria metabolizing middle distillate and crude oils. The oil microbiocidal data will be discussed with emphasis on the effect of the oil versus water solubility of quaternary salts.

1. Oil Versus Water Solubility

The oil versus water solubility of quaternary higher monoalkyl versus dialkyl ammonium salts was studied in this work using the common coco ammonium chlorides, previously discussed. As partition media heating oil and distilled water were selected.

The heating oil used had about 90% paraffinic and 10% aromatic hydrogens, according to proton magnetic resonance spectroscopy (pmr). It was substantially free from olefinic components. This heating oil was mostly used throughout this study.

The preferential water solubility of the monococo trimethyl ammonium chloride was, of course, already known. Similarly, the dicoco dimethyl ammonium chloride was previously considered as an "oil soluble" quaternary salt. However, no quantitative data were available on their relative oil versus water solubilities.

In the determination of the oil versus water distribution of the above quaternary salts and other organo-nitrogen biocides, 0.2 g of the additive was used in a 1:1 mixture with ethanol. This was added to 100 ml of the oil and the resulting oil to 100 ml water. The mixture was then magnetically stirred in an Erlenmeyer flask at about 50 rpm for 2 hours. Thereafter, a 4 ml sample of the water phase was taken by a pipette for nitrogen analyses. Similar distribution experiments were carried out using the coco ammonium chlorides and an α -methyl branched alkyl trimethyl ammonium chloride, the latter having a roughly equal distribution of C₁₁ to C₂₀ alkyl groups. Grotan, i. e. tris-hydroxymethyl hexahydrotriazine, was employed as a commercial non-quaternary microbiocide for comparison. The results are shown by Table II. They indicate that, as expected, the monococo compound is mostly concentrated in the water phase while the dicoco derivative is more soluble in oil than in water.

2. Microbiocidal Effects

The oil microbiocidal effects of quaternary higher alkyl trimethyl ammonium chlorides of varying chain length were the primary objects of study in this work. In most of the tests, bacteria isolated from the water bottoms of home heating oil tanks were used as test organisms under various experimental conditions.

In the first series of experiments shown by Table IV, the comparative bactericidal effectiveness of trimethyl ammonium chlorides higher n-alkyl groups of different chain length was investigated in a water medium containing all the essential salts for their growth, i. e. a P₁ basal salt medium (14). The composition of this medium is shown in Table III.

The test procedure used a gram negative bacterial strain isolated from a home oil heater. The organisms were grown in the presence of 20, 100 and 500 ppm of quaternary salt. In each test, 10 ml of basal salt medium overlaid with 0.1 ml, i. e. 1% of diesel fuel was used. Diesel fuel rich in alkanes was employed as a preferred fuel nutrient. After a 48 hour growth period, samples were streaked on nutrient agar plates and incubated for 48 hours at 30°C to determine the viability of the test organisms. The test mixtures were sampled after 24 and 48 hours to obtain the data shown in Table VII.

The data show that, in general, the mixtures of homologous alkyl compounds (Groups II and III) showed better inhibition than the pure alkyl compounds (Group I). Furthermore, the detergent range alkyl compounds (Nos. 3 and 5) were more active than those having even higher alkyl groups (Nos. 4 and 6). Of the higher monoalkyl compounds the more water soluble C₁₄-alkyl derivative is more effective than the C₁₈ compound although the latter has a smaller critical

micelle concentration (15) and is more effective in reducing the interfacial tension of water-Nujol hydrocarbon systems (16).

Interestingly, no difference was observed in the activity of the 1- and 2- alkyl detergent range compounds (Nos. 3 and 5). Both the completely straight chain (No. 3) and the alpha methyl branched compounds (No. 5) completely inhibited bacterial growth for 48 hours at 20 ppm.

In the second series of experiments (Table V), the effect of various concentrations of n-dodecyl trimethyl ammonium chloride on the growth of various bacterial strains utilizing jet fuel was studied.

Five contaminated jet fuel samples were used to isolate five bacterial strains by the spread plate technique. The isolated strains were further purified and then characterized as rod-like gram negative organisms.

In the test, the five bacterial isolates were routinely maintained on nutrient agar slants by weekly transfers. The above five cultures (24 hr. old slant cultures) were inoculated into culture tubes containing 7 ml of sterile nutrient broth and incubated at 30°C under stationary conditions for 24 hours. At the end of the incubation period, contents from each tube were transferred under sterile conditions into sterilized centrifuge tubes. The cells were harvested by centrifugation of 10,000 rpm for 15 minutes. The cells were washed by resuspending the pellet in 10 ml sterile P₁ basal salts medium and recentrifuging. The washed cells were resuspended again in 10 ml of sterile medium. One ml from each suspension containing approximately 10³ cells/ml was used as the inoculum to each flask containing 9 ml of basal salts medium and the appropriate concentration of the inhibitors. Finally each flask was layered with 5 ml of Millipore filter sterilized jet fuel. The inoculated flasks were incubated for 40 hours on a New Brunswick rotary shaker maintained at 30°C and rotating at 250 rpm.

The growth in flasks was read at the end of seven days of incubation. At that time, a 0.01 ml sample from each flask was streaked on nutrient agar plates and the plates were incubated overnight at 30°C to determine the viability of the test microorganisms.

The test results, shown by Table V, indicate that, at 500 ppm, n-dodecyl trimethyl ammonium chloride completely inhibited for a week the growth of all the bacterial strains.

It should be noted that in commercial use, prevention of troublesome bacterial growth rather than total inhibition is sufficient. For this reason and due to the excess basal salt layer, the concentration of the water soluble salt additives would be smaller in field use than in these laboratory tests.

In the third series of experiments (Table VI), quaternary higher mono- and dialkyl ammonium chlorides derived from coconut oil were compared for their bactericidal effectiveness against a mixture of bacteria.

A heating oil was employed as a hydrocarbon phase. As component of the water phase, the P₁ basal salt medium of the previous test series was used. The bacterial test organisms were isolated from tank bottoms of two home oil heaters. They consisted of three gram negative rod-shaped bacteria and a gram positive spore-forming bacillus.

The mixed bacterial cultures were carried on trypticase soy agar slants supplemented with a drop of the heating oil. To prepare the test, inoculum, a 48 hour slant culture, was washed off with 10 ml of the P₁ basal salt solution and added to a mixture 100 ml of the P₁ solution, 1 ml of 10% aqueous peptone and 1 ml of heating oil. This culture was incubated at room temperature for six days while being agitated by a magnetic stirrer. At the end of this incubation period, the total bacterial count had risen from 1 x 10⁷ per ml to 1 x 10⁸ per ml.

In a modified simulated tank bottom test, the quaternary salt was added as a 0.1 ml of ethanolic solution to 100 ml agitated heating oil. While maintaining hand agitation, 4 ml P₁ solution and 1 ml culture were also added. The assays were performed in serum bottles of 2 in. in diameter and 4 in. high. The test bottles were closed with rubber stoppers and vented through a #18 gauge hypodermic needle. They were incubated for 7 days under stationary conditions on a laboratory bench at about 24°C.

Dependent on the chemical structure and the concentration of the quaternary salt employed, various degrees of bacterial growth were observed in the aqueous phase and at the interface.

In another variation of this series of tests, instead of 100 ml heating oil, only 1 ml heating oil was used in a serum bottle.

In both types of tests, samples of the mixtures were taken after 7 days, as follows. After gentle swirling for homogenization, a 0.2 ml sample was withdrawn from the interface region of the contents of the flask or bottle using a 1 ml tuberculin syringe fitted with an 18# gauge needle. The sample was then serially diluted in sterile water. Plate counts were performed on trypticase soy agar incubated at 35°C for 48 hours. The data on the reduction of the number of viable cells obtained after 7 days with both methods are shown by Table VI.

The results indicate that in the test using the excess oil the preferably water soluble monococo trimethyl ammonium chloride was about 10 times more effective than the preferably oil soluble dicoco dimethyl ammonium chloride. A four log reduction of bacterial population from

the order of 10^8 to 10^4 is effected by about 0.3 mg of the monococo compound in the presence of 100 ml oil. Under the same conditions the same reduction is effected by about 3 mg of the dicoco compound.

In contrast, the same monococo and dico compounds show about the same higher order of effectiveness if little oil is present. With 100 ml heating oil added, it takes only about 0.3 mg of both the higher mono- and the higher dialkyl compound to produce the same 4 log reduction from the order of 10^8 to 10^4 organisms.

In the fourth series of experiments (Table VII), the comparative effectiveness of the preferably water soluble tetradecyl ammonium chloride and of the oil soluble dicoco dimethyl ammonium chloride was determined against aerobic bacteria isolated from crude oil from Block 73, Louisiana. The test system was a 3.5% NaCl aerobic broth bacteria culture containing 100,000 bacteria per milliliter. Different concentrations of the quaternary salts were added to vials containing the broth medium. The media were then inoculated. The vials were incubated at 30°C and observed at intervals for evidence of bacterial growth. The results are shown by Table VII.

The data showed that about 50 ppm monoalkyl compound was required for complete inhibition while 100 ppm dialkyl derivative was necessary for the same effect.

In the last series of experiments (Table VIII), the effectiveness of several higher monoalkyl trimethyl ammonium chlorides was determined against sulfate reducing, anaerobic bacteria in comparative tests with a commercial higher dialkyl dimethyl ammonium chloride. The test procedure was the same as in the previous test series. The bacterial strain was isolated from the Borregas Field, Kingsville, Texas.

The data show a complete growth inhibition for a minimum of 14 days by all the quaternary ammonium chlorides at the 50 ppm level. At 25 ppm the higher mono-coco compound still shows a complete inhibition. The corresponding dicoco derivative shows incomplete control at this concentration. The best compound appears to be tetradecyl trimethyl ammonium chloride which provides complete inhibition at 12 ppm.

CONCLUSIONS

Preferably water soluble, C₁₂ to C₂₀ alkyl trimethyl ammonium chlorides appear attractive as heating oil microbiocide candidates. Among the n-alkyl compounds the tetradecyl compound and the coco derivative are about the most effective as bactericides.

The oil bactericidal activity of structurally related, preferably oil soluble quaternary ammonium salts, having two C₁₂ to C₂₀ alkyl groups, is mostly inferior to the higher monoalkyl compounds. The dicoco compound is much inferior to the monococo derivative in systems containing a high volume of oil and a relatively low volume of water.

Preferential water solubility for quaternary oil microbiocides appears attractive because concentration in the relatively small volume water bottoms of oil tanks will assure a higher concentration of biocidal compounds at the water side of the interface, where microorganisms proliferate. If the solubility preference for water is moderate, loss of the additive is usually no problem.

ACKNOWLEDGMENTS

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