N-(1,1-dihydroperfluorooctyl)pyridinium Trifluoromethanesulfonate, a New Quaternary Ammonium Antiseptic

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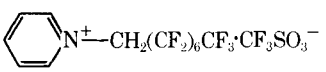
Abstract □ The synthesis and biological evaluation of *N*-(1,1-dihydroperfluorooctyl)pyridinium trifluoromethanesulfonate (MBR 3092-42), a highly fluorinated quaternary ammonium antiseptic, are described. The material is structurally related to cetylpyridinium chloride. It shows approximately equivalent bacteriostatic activity to cetylpyridinium chloride but somewhat less fungistatic activity. The bactericidal activity of MBR 3092-42 is slightly less than that of cetylpyridinium chloride in equal concentration in distilled water or in 10% blood serum, but is slightly greater in 80% human blood. MBR 3092-42 is also antibacterially equivalent to cetylpyridinium chloride on the resident bacterial flora of human skin. Although this new compound slowly decomposes in water at pH 7 and above, it is stable in weakly acidic aqueous or hydroalcoholic solutions. Solutions of MBR 3092-42 yield much lower surface tension values than equivalent concentrations of cetylpyridinium chloride.

Keyphrases □ *N*-(1,1-dihydroperfluorooctyl)pyridinium trifluoromethanesulfonate (MBR 3092-42)—synthesis □ Antimicrobial activity—MBR 3092-42 □ Dermal toxicity—MBR 3092-42 □ Acute toxicity—MBR 3092-42 □ Irritation, sensitivity testing—MBR 3092-42

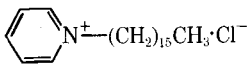
The quaternary ammonium-type germicides, introduced by Domagk (1), are highly effective broad spectrum germicides and fungicides. However, this class of compound suffers from the disadvantage of being inactivated by lecithin, long-chain fatty acids, and other anionic materials. In spite of this, they have been successfully used as antiseptics for general and surgical applications for many years.

In general, quaternary ammonium germicides are nontoxic and nonirritating to skin and mucous membranes at the concentrations used for antiseptic effects (2). At higher concentrations, however, these compounds are toxic and irritating to skin and mucous membranes. Most commercially available compounds of this class show similar toxicological and pharmacological properties (3–6).

This report concerns itself with the synthesis and biological properties of MBR 3092-42, and compares these properties, in most cases, with those of cetylpyridinium chloride, one of the oldest and most active of the quaternary ammonium germicides currently in use.



MBR 3092–42



cetylpyridinium chloride

EXPERIMENTAL

Synthesis—*N*-(1,1-dihydroperfluorooctyl)pyridinium trifluoromethanesulfonate. 1,1-Dihydroperfluorooctyl trifluoromethanesulfonate (7) (1.30 kg., 2.44 moles) was added over 2 hr. to a solution of 2.59 kg. (3.06 moles) of pyridine in 2.1 l. of acetone. After the solution was warmed at 40° for 24 hr., the product was precipitated by cooling and addition of excess isopropyl ether. The collected solid was purified by reprecipitation from acetone–ether to afford 870 g. (58%) of product, m.p. 112.5–114.5°.

Antimicrobial Activity—Bacteriostatic and fungistatic activities were compared using the filter paper disk-agar plate diffusion assay method of Vincent and Vincent (8). Activity was determined both in the presence and absence of blood or serum. Experiments were carried out at three dosage levels: 40, 4, and 0.4 mcg. per paper disk on PGY agar:

PGY Agar	Concentration, g./l.
NaCl	8.0
KCl	0.4
$MgSO_4 \cdot 7H_2O$	0.154
$CaCl_2 \cdot 7H_2O$	0.016
$Na_2HPO_4 \cdot 7H_2O$	0.29
KH_2PO_4	0.15
Phenol Red	0.6 ml. of 0.2% soln.
Yeast extract	1.0
Glucose	2.0
Ionagar	8.5
Deionized water to 1 l.	

Bactericidal properties were assayed using a quantitative kill-rate method similar to that originally described by Chick (9). The test was carried out in three media: deionized water, 10% horse serum, or 80% human blood in deionized water. The test medium was inoculated with the test microorganisms at a rate of approximately 10^5 cells per ml., and the test chemical was added. Solutions were prepared of the test chemicals at 1% in 20% alcohol (necessary to aid in solubilizing MBR 3092-42), and serial dilutions were prepared in deionized water out to extinction of activity. Dilutions of 1–10 or higher were found not to contain sufficient alcohol to have significant antibacterial activity. At intervals of 1, 5, 30, and 120 min., portions were removed and diluted serially in a dilution medium containing lecithin and polysorbate 80[1] to neutralize the germicide.

	Concentration, g./l.
KH_2PO_4	6.80
$K_2HPO_4 \cdot 3H_2O$	11.4
$Na_2S_2O_3 \cdot 5H_2O$	5.0
Lecithin	2.5
Polysorbate 80	15.0
Deionized water to 1 l.	

Plate counts were then made using standard methods agar.

The ability of the compounds to kill the resident bacteria on human skin was compared using a serial skin-stripping method as described by Updegraff (10). For these studies, MBR 3092-42 and cetylpyridinium chloride[2] were dissolved in water at 1.0%. Solution of MBR 3092-42 was accomplished by warming in a water bath to 40 to 50°, and the solution was kept warm in this bath during the test to prevent precipitation of the compound. Dilutions were made in deionized water to 0.1 and 0.01%. These were not warmed

[1] Tween-80, Atlas Chemical Industries, Wilmington, Del.

[2] The cetylpyridinium chloride used in this and other studies was purchased from Calbiochem.

in the case of MBR 3092-42 as there was no tendency for the compound to precipitate at the low concentrations. Test solutions were applied to areas of the skin on the plantar surface of the forearm of three experimental human subjects and allowed to remain for 30 min. Residual germicide was washed off with sterile deionized water and the skin was gently patted dry with sterile gauze. Cultural counts were then made for bacteria in sequential layers of skin.

Effects of MBR 3092-42 in Anesthetized Dogs—Mongrel dogs were anesthetized with intravenous pentobarbital sodium, 30 mg./kg., and both vagus nerves were sectioned in the midcervical region. Blood pressure was recorded directly from the femoral artery by means of a P23AA Statham pressure transducer. Respiration was recorded *via* a bellows pneumograph fastened around the chest and connected to a P23BB Statham pressure transducer. A Lead II electrocardiogram was recorded to monitor heart rate. All parameters were recorded on a Type R Beckman Dynagraph. Drugs were administered intravenously in the indicated doses directly into the femoral vein.

Acute Toxicity—Determinations of the acute LD_{50} were carried out in rats and mice by both the oral and intraperitoneal routes. Calculations of the LD_{50} values were accomplished by the method of Miller and Tainter (11).

Mice—Simonsen Swiss Webster male mice weighing 18–30 g. were used in all studies. The compounds were administered either orally or intraperitoneally suspended in 4% acacia. Ten mice were used at each dosage level and were group-housed by dosage level in solid bottom plastic cages with food and water available *ad libitum*. The mice were not fasted prior to compound administration. Animals were observed for pharmacodynamic signs and mortality for several hours postdosage and daily thereafter for 14 days.

Rats—Simonsen Sprague Dawley female rats (150–200 g.) were used for all studies. The compounds were administered as a suspension in 4% acacia to fasted animals by either the oral or intraperitoneal route. Eight rats were used for each dosage level and were group-housed by dosage level in suspended screen bottom cages with food and water available *ad libitum*. The rats were observed for pharmacodynamic signs and mortality for several hours after dosing and daily for 14 days thereafter.

Other Species—Oral lethality studies were carried out in dogs and cats by administration of the compound in gelatin capsules to fasted animals of unspecified sex.

Acute Dermal Toxicity—Albino rabbits weighing initially 1630 to 2314 g. were used in this study. The animals were divided into seven groups of four rabbits (two males and two females) each. An area of the back corresponding to approximately 10% of the body surface was prepared by close clipping with an electric clipper. Additionally, one male and one female rabbit in each group were abraded over this area by producing shallow incisions with a scalpel blade. A 10% concentration of the test compound in 70% ethanol was applied to the backs of the rabbits by repeated applications of 4–5 ml. until the animals had received the proper dosage. The area was then covered with surgical gauze which was held in place by wrapping the body with an elastic bandage. Application of compounds was carried out once only at dosage levels of 0.5, 1.0, and 2.0 g./kg. The seventh group of four rabbits was treated with 70% ethanol only in a volume equivalent to that applied to the 2.0 g./kg. group for control purposes.

All of the animals were immobilized for 24 hr. in stocks. After this restraint period the compound was washed off with tap water and animals were observed for dermal irritation. The animals were the nhoused individually for a 14-day observation period in suspended metal cages. Hematologic examinations and urinalysis were conducted once during the control period and at 24 hr. and 2 weeks after compound administration. Daily observations were made for mortality, food consumption, systemic toxicity, and dermal irritation. Dermal irritation was scored as outlined by Draize (12).

Irritation Tests—Rabbits were utilized for eye irritation assay as follows. Albino rabbits of unspecified sex were placed in a restraining stock until quiet. The test materials in varying concentrations (0.5, 1.0, and 2.0%) were then instilled into the right eye in 0.1-ml. amounts. Isotonic saline was instilled into the left eye which served as control. The lids were gently held together for 1 sec. and then released. The eyes were not washed following instillation of the test material. Two rabbits were utilized for each concentration of material tested. The eyes were examined for degree of irritation and scored at intervals as described under Section 191.12 of the Federal Hazardous Substances Labeling Act Regulations (13). The lowest concentration producing signs of irritation considered positive under these regulations (ulceration or opacity of cornea, inflammation of iris, or swelling or redness of the conjunctiva) was considered to be the threshold irritation concentration.

Table I—Surface Tension of Aqueous Solutions Determined Using a DuNoüy Ring and Instron Tester

Compound	Concentration, % w/v	Surface Tension, dynes/cm.
MBR 3092-42	0.05	34.0
MBR 3092-42	0.10	26.0
Cetylpyridinium chloride	0.05	42.0
Cetylpyridinium chloride	0.10	45.5
Water	—	76.0

Primary dermal irritation was determined utilizing guinea pigs. Twelve male albino guinea pigs weighing from 340 to 441 g. were used in this study. The dorsal hair was removed with an electric clipper over an area of approximately 5.08 cm. (2 in.) square. The animals were separated into two groups and the skin of one-half the guinea pigs in each group was further prepared by abrading the shaved area with a scalpel blade.

The prepared skin was dampened with normal saline, and 0.1 g. of the test compound was then placed on the prepared area of six guinea pigs (three with intact and three with abraded skin). The area was covered with gauze and the body of each animal wrapped with elastic bandage. Twenty-four hours later the bandages were removed and the areas of application washed with tepid tap water and examined for dermal irritation. Seventy-two hours later the areas were again examined. Scoring of irritation was as outlined by Draize (12).

Sensitization Testing—Testing for dermal sensitization was performed on male albino guinea pigs. Twenty guinea pigs weighing from 260 to 336 g. were used for this study. The animals were individually housed in suspended metal cages with food and water available *ad libitum*. The laboratory food[3] diet was supplemented with fresh cabbage three times weekly.

The test compounds and the positive control (2,4-dinitro-1-chlorobenzene) were dissolved in sterile distilled water at 0.1% concentration just prior to injection. The back of each guinea pig was prepared by close shaving with an electric clipper. This was repeated as necessary throughout the study.

The positive control and test compounds were injected intradermally (using a 26-gauge needle) into the prepared area every other day, until a total of 10 such sensitizing doses had been given. The volume of the first dose was 0.05 ml. and that for the remaining doses was 0.10 ml. No injection site was used more than once.

Two weeks after the final sensitizing dose had been administered, a challenge dosage of 0.05 ml. of the respective test and positive control compounds was administered intradermally.

The injection sites were examined for diameter, height, and intensity of erythema and edema 24 and 48 hr. following each individual injection. Results were scored as follows: 0.0, normal; 0.5, trace (faint pink); 1.0, pink; 2.0, red; 3.0, yellow-pink; 4.0, yellow (necrosis).

The 24- and 48-hr. readings were averaged and means calculated. Similar measurements were made 24 and 48 hr. after the challenge dosages and averaged.

RESULTS

Analysis of the synthetic product obtained by the procedure described was as follows:

Anal.—Calcd. for $C_{14}H_7F_{18}NO_3S$: C, 27.53; H, 1.15; F, 55.92; N, 2.30. Found: C, 27.58; H, 1.29; F, 55.87; N, 2.37.

Physical and Chemical Properties—MBR 3092-42 is a white, odorless, crystalline compound, with a bitter taste and somewhat less aftertaste than most quaternaries. In comparative taste tests with cetylpyridinium chloride, it was somewhat less bitter and was judged to have less aftertaste.

It is soluble in water to 0.1% (w/v); ethanol, 24.0%; acetone, 53.0%; hexane, 0.7%; and polyethylene glycol 400, 3.0%. MBR 3092-42 is very effective at lowering the surface tension of water

[3] Purina Laboratory Chow.

Table II—Antimicrobial Activity of MBR 3092-42 and Cetylpyridinium Chloride as Measured by Agar Plate Diffusion

Test Organism	MBR 3092-42 — Diameter of Zone of Inhibition, mm. — Serum Free: 40 mcg.	4 mcg.	0.4 mcg.	MBR 3092-42 — With 10% Horse Serum: 40 mcg.	4 mcg.	0.4 mcg.	Cetylpyridinium Chloride — Serum Free: 40 mcg.	4 mcg.	0.4 mcg.	Cetylpyridinium Chloride — With 10% Horse Serum: 40 mcg.	4 mcg.	0.4 mcg.
Staphylococcus aureus (FDA 209)	29	16	x[a]	25	13	x	18	11	x	15	11	7
Streptococcus sp. 104[b]	18	10p[c]	x	24	14	x	16	12	x	14	12	9
Escherichia coli (Gratia)	25	x	x	21	x	x	16	10	x	10	x	x
Pseudomonas aeruginosa	x	x	x	x	x	x	8	x	x	x	x	x
Bacillus subtilis	51	35	17	32	21p	10p	26	16	8	17	12	8
Aspergillus niger	13p	x	x	15p	x	x	18	11	2	14	9p	x
Candida albicans	13p	x	x	10	x	x	19	11	x	12	x	x

[a] x = no activity. [b] A species isolated from human dental caries. [c] p = partial inhibition in the zone.

Table III—Bactericidal Activity of MBR 3092-42 and Cetylpyridinium Chloride as Determined in Deionized Water or 10% Horse Serum

Germicide	Test Medium	Kill After 2 hr., % — Dilution of Test Compound — *S. aureus* 1/10	1/100	1/1000	1/10000	1/100000	*E. coli* 1/10	1/100	1/1000	1/10000	1/100000	*P. aeruginosa* 1/10	1/100	1/1000	1/10000	1/100000
MBR 3092-42, 1% in 20% alcohol	Water	100	100	100	83	82	100	100	100	58	0	100	100	99	93	0
MBR 3092-42, 1% in 20% alcohol	10% Horse serum	100	100	0	0	0	100	90	23	0	0	99	84	0	0	0
Cetylpyridinium chloride, 1% in 20% alcohol	Water	100	100	100	100	92	100	100	100	100	51	100	100	100	97	0
Cetylpyridinium chloride, 1% in 20% alcohol	10% Horse serum	100	100	0	0	0	100	100	30	0	0	100	100	36	0	0

(Table I) and it is unique in that the concomitant foaming property frequently seen with surfactants is lacking.

Like cetylpyridinium chloride, MBR 3092-42 is decomposed by alkali, producing a brown or yellow solution or precipitate devoid of antibacterial activity, as shown by complete lack of any inhibition zones at the highest test dose, 40 mcg., against any of the test organisms listed in Table II.

Antimicrobial Activity—The bacteriostatic and fungistatic activities of MBR 3092-42 and cetylpyridinium chloride are compared in Table II. From these data, we can conclude that both agents are powerful bacteriostatic agents and moderately potent fungistatic agents.

The bactericidal activities of these agents toward *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* are presented in Table III and Figs. 1 and 2. It is clear that both agents are powerfully bactericidal. Cetylpyridinium chloride appears to be slightly more active in water and 10% horse serum as indicated by Table III, but when tested in 80% human blood, MBR 3092-42 showed superior activity against both *S. aureus* and *E. coli*, as shown in Figs. 1 and 2.

The ability of MBR 3092-42 to kill the resident flora of the human skin, as compared with cetylpyridinium chloride, is presented in Table IV. The first two experiments, carried out on subjects DMU and RMW, showed complete kills in several cases with 1% solutions of the test materials; therefore, lower concentrations were used in subsequent tests. Control counts were well within the range reported for normal human subjects (10). Both agents are highly effective and comparable in activity within experimental error reported for this procedure (10).

Effects in Anesthetized Dogs—When administered intravenously to anesthetized dogs as single doses, neither MBR 3092-42 nor cetylpyridinium chloride produced marked effects on heart rate, blood pressure, and respiration until the toxic dose level was reached (Table V). High doses of either agent produced bradycardia and a fall in blood pressure. The fatal single doses of MBR 3092-42 and cetylpyridinium chloride were found to be 20 mg./kg. and 40 mg./kg., respectively. Death in both cases was apparently due to res-

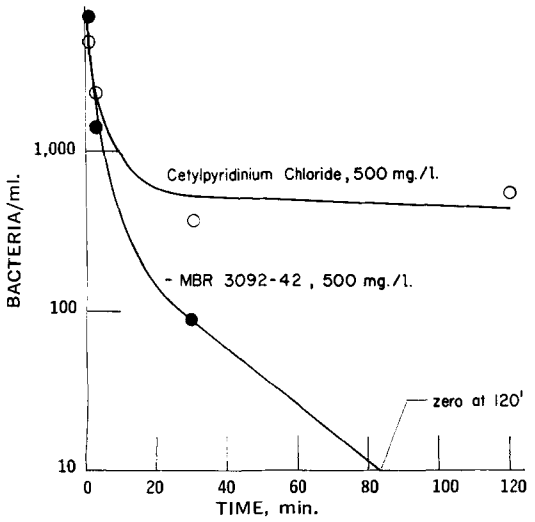


Figure 1—*Kill-rate curves of* Escherichia coli *by MBR 3092-42 and cetylpyridinium chloride in 80% human blood.*

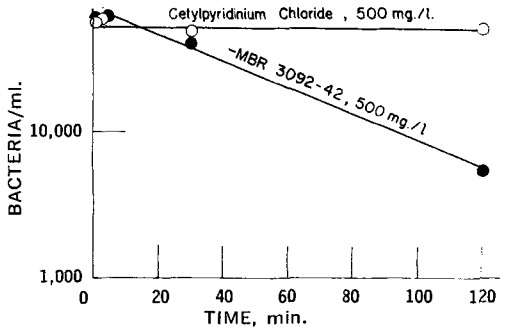


Figure 2—*Kill-rate curves of* Staphylococcus aureus *by MBR 3092-42 and cetylpyridinium chloride in 80% human blood.*

Table IV—The Germicidal Activity of MBR 3092-42 and Cetylpyridinium Chloride Against Human Skin Bacteria Determined by a Serial Skin-Stripping Method

Treatment	Concn.	Test Subject	Bacterial Count per 16 cm.² of Skin[a] 1	2	3	4	5	6	Total	Kill, %
Control		DMU	650	560	350	400	192	116	2268	—
C.P. Cl[b]	1%	DMU	5	2	0	0	0	0	7	99.7
3092-42	1%	DMU	3	0	0	0	0	3	6	99.7
Control		RMW	135	56	45	27	20	8	291	—
C.P. Cl	1%	RMW	7	1	0	0	0	1	9	97.0
3092-42	1%	RMW	1	0	3	0	0	1	5	98.3
Control		RMW	100	150	—	56	43	31	380	—
C.P. Cl	0.1%	RMW	10	5	7	7	2	10	41	89.0
C.P. Cl	0.01%	RMW	7	7	6	7	7	8	41	89.0
3092-42	0.1%	RMW	8	6	3	1	2	3	23	94.0
3092-42	0.01%	RMW	11	15	12	6	8	5	57	85.0
Control		VSP	150	46	107	111	95	105	624	—
C.P. Cl	0.1%	VSP	0	0	0	1	1	0	2	99.7
C.P. Cl	0.01%	VSP	14	19	8	13	5	8	67	89.0
3092-42	0.1%	VSP	2	2	1	4	3	1	13	97.9
3092-42	0.01%	VSP	30	28	31	12	35	25	161	74.0

[a] Each value represents the bacterial count obtained from each 16 cm.² layer of skin, the higher numbers representing serially deeper layers. [b] Cetylpyridinium chloride.

piratory failure. Continuous intravenous infusion of these agents at constant rates produced death at an average cumulative dose of 88 mg./kg. with cetylpyridinium chloride and at an average cumulative dose of 97 mg./kg. with MBR 3092-42 (Table VI). Death in both cases was due to respiratory failure preceded by marked bradycardia and hypotension. MBR 3092-42 was found to cause greater depression of heart rate and blood pressure than cetylpyridinium chloride at doses below the toxic level.

Table V—Effect of Single Intravenous Injections of MBR 3092-42 or Cetylpyridinium Chloride on Heart Rate, Blood Pressure, and Respiration in Anesthetized Dogs[a]

Compound	Dose, mg./kg.[b]	Maximum Change, % Heart Rate	Blood Pressure	Respiration
Cetylpyridinium chloride	1	−5	0	0
	3	0	−5	0
	10	0	+5	0
	20	+5	−30	0
	40		Fatal[c]	
MBR 3092-42	1	0	−5	+5
	3	0	−10	0
	10	−15	−25	0
	20		Fatal[c]	

[a] MBR 3092-42 was administered as a 1% solution in distilled water acidified with 0.1 *N* HCl sufficient to solubilize the compound. Cetylpyridinium chloride was administered as a 4% solution in distilled water. [b] One dog was used for each dosage level administered. [c] Respiration ceased shortly after drug administration with death following from respiratory failure. Bradycardia and hypotension were noted immediately preceding respiratory arrest.

The mechanism for the toxic effect of cetylpyridinium chloride has been found to be a neuromuscular blockade with subsequent paralysis of the muscles of respiration (3). From these experiments it would appear that a similar mechanism is also responsible for the toxic effect of MBR 3092-42.

Acute Toxicity—The acute LD_{50} values obtained are summarized in Table VII. MBR 3092-42 proved to be less toxic than cetylpyridinium chloride in rats and mice by most routes, with the exception of the intraperitoneal route in rats. Administration of either compound to dogs or cats produced emesis of such severity as to preclude determination of lethal potential in these species by the oral route.

Results of the acute dermal toxicity studies in rabbits are reported in Table VIII. The degree of dermal irritation produced was much more prominent in those rabbits receiving cetylpyridinium chloride than in those receiving MBR 3092-42. Rabbits receiving cetylpyridinium chloride exhibited erythema, edema, atonia, desquamation, leathery texture, fissuring, necrosis, ulceration, and sloughing of the skin. These signs bore a dose relationship, particularly in relation to the necrosis, sloughing, and ulceration. Rabbits administered MBR 3092-42 exhibited erythema, edema, and atonia of the skin over a 24–48-hr. period following application. However, the skin appeared essentially normal thereafter.

Control rabbits and rabbits administered MBR 3092-42 exhibited essentially normal values in hematology and urinalysis during this study. Rabbits receiving cetylpyridinium chloride exhibited an elevation in nonsegmented neutrophils at the 24-hr. period after treatment which was dose related in severity.

No unusual alterations in body weight or food consumption were noted in animals receiving MBR 3092-42. Rabbits administered cetylpyridinium chloride exhibited partial anorexia for the first week after treatment. This appeared to be dose related and was accompanied by loss of body weight, particularly at the 2.0 g./kg. dose level.

Irritation Testing—In ocular irritation studies in rabbits, the threshold irritation concentrations were found to be 0.5% for cetylpyridinium chloride and 1.0% for MBR 3092-42.

Dermal irritation studies in guinea pigs over a 72-hr. period produced no evidence of skin irritation under the conditions of the

Table VI—Effect of Intravenous Infusion of MBR 3092-42 and Cetylpyridinium Chloride on Heart Rate, Blood Pressure, and Respiration in Anesthetized Dogs

Dog No.	Compound	Infusion Rate, mg./kg./min.[a]	Fatal Dose, mg./kg.	Maximum Change, % Heart Rate	Blood Pressure	Respiration
1	Cetylpyridinium chloride	1.0	81	−67	−73	Progressive depression to failure
2	MBR 3092-42	1.0	90	−23	−38	Progressive depression to failure
3	Cetylpyridinium chloride	2.0	98	−24	−50	Progressive depression to failure
4	MBR 3092-42	2.0	104	−69	−91	Progressive depression to failure

[a] Drugs were infused as solutions in distilled water at a concentration of 3 mg./ml.

Table VII—Acute Toxicity of MBR 3092-42 and Cetylpyridinium Chloride

Test Species	Route	LD_{50}, mg./kg. and *SE* MBR 3092-42	Cetylpyridinium Chloride
Mouse, male	Oral	925 ± 45.2	175 ± 14.6
Mouse, male	i.p.[a]	30.0 ± 1.4	7.0 ± 0.6
Rat, female	Oral	1159 ± 141.3	538 ± 49
Rat, female	i.p.[a]	18.0 ± 1.0	26.5 ± 1.3
Cat	Oral	Emetic[b]	Emetic[b]
Dog	Oral	Emetic[b]	Emetic[b]

[a] Intraperitoneal. [b] Oral doses of 250 and 500 mg./kg. (two animals/dose level) to dogs or cats produced emesis and no observable toxic signs. The emesis prevented establishment of a minimal lethal dose.

Table VIII—Acute Dermal Toxicity of MBR 3092-42 and Cetylpyridinium Chloride in Albino Rabbits

Compound	Dermal Dose, g./kg.	Mortality No. Dead/ No. Tested	Irritation No. Positive[a]/ No. Tested
70% Ethanol	—	1/4[b]	0/4
Cetylpyridinium chloride	0.5	0/4	4/4
	1.0	0/4	4/4
	2.0	2/4[c]	4/4
MBR 3092-42	0.5	0/4	2/4
	1.0	0/4	4/4
	2.0	0/4	4/4

[a] Positive animals were those showing any signs of irritation. [b] One rabbit died from a broken neck while restrained in the stock. [c] One rabbit (abraded) died on Day 4 and one (intact) on Day 5.

experiment with either MBR 3092-42 or cetylpyridinium chloride. Due to the negative scores obtained, no table of results is presented.

Sensitization Testing—The results of a dermal sensitization study conducted in guinea pigs are presented in Table IX. These indicate that the test compounds, cetylpyridinium chloride and MBR 3092-42, produced irritation since wheal and flare formation was evident throughout all 10 sensitizing injections. The values obtained from the challenge dosage were similar to the average values obtained with the sensitizing injections. Dinitrochlorobenzene as the positive control also produced evidence of irritation. However, the values obtained from the challenge dosage exceeded those obtained from the sensitizing injections. Thus, it is concluded that MBR 3092-42 and cetylpyridinium chloride produced irritation but not sensitization and that dinitrochlorobenzene produced sensitization as well as irritation.

DISCUSSION

The antibacterial activity of quaternary compounds is believed to be correlated with their surfactant properties (2). Fluorine substitution markedly increases the surface activity of many surfactants, as measured by surface tension reduction (14). This property could account for the high bactericidal activity of MBR 3092-42 as contrasted with its nonfluorinated homolog *n*-octylpyridinium chloride, which has very slight activity (15).

On the other hand, the introduction of fluorine decreases water solubility and increases formula weight to a value of 611 for MBR 3092-42 *versus* 358 for cetylpyridinium chloride. Thus, on a molar basis, MBR 3092-42 is proportionately more active than cetylpyridinium chloride.

The relative lack of toxicity and irritation to the skin and mucous membranes with MBR 3092-42 and its effectiveness in the presence of blood would appear to be advantageous for its use as an antiseptic for the skin and surgical wounds. Further, its high surface activity and lack of foaming properties as well as its somewhat less unpleasant taste should prove advantageous for other uses, *e.g.*, as an oral antiseptic. For this purpose, dilute hydroalcoholic solutions could conceivably be preferable to weakly acidic solutions, since acidic solutions might aggrevate the formation of dental caries.

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Table IX—Studies on Dermal Sensitization in the Guinea Pig with Cetylpyridinium Chloride, MBR 3092-42 and 2,4-Dinitro-1-chlorobenzene

Test Compound	No. Guinea Pigs	Reaction Scores, Mean ± *SE*: Sensitizing Doses, Diameter, mm.	Sensitizing Doses, Height mm.	Sensitizing Doses, Color[a]	Challenge Dose, Diameter, mm.	Challenge Dose, Height, mm.	Challenge Dose, Color[a]
2,4-Dinitro-1-chlorobenzene	4	9.2 ± 0.3	1.2 ± 0.1	2.1 ± 0.1	14.2 ± 0.1	1.4 ± 0.2	2.3 ± 0.2
Cetylpyridinium chloride	7[b]	8.9 ± 0.2	0.8 ± 0.04	2.7 ± 0.05	9.7 ± 0.03	0.4 ± 0.1	3.0 ± 0.1
MBR 3092-42	8	8.6 ± 0.1	1.1 ± 0.05	2.4 ± 0.1	9.7 ± 0.4	0.9 ± 0.1	2.3 ± 0.1

[a] 0 = normal, 0.5 = trace, 1 = pink, 2 = red, 3 = yellow-pink, 4 = yellow. [b] One animal died during the final week of the study.