

# A New Broad Spectrum Antibacterial Soap

## I. General Properties<sup>1</sup>

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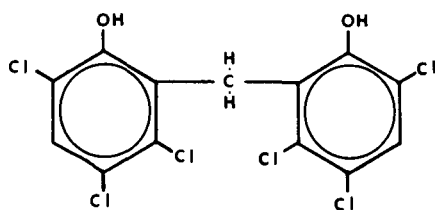
### ABSTRACT

The first soap germicide system to show activity against gram-negative organisms is described. Addition of a new germicidal agent, 2-hydroxy-2',4,4'-trichlorodiphenyl ether, combination of hexachlorophene and triclocarban which has been used in a soap known to be effective against gram-positive organisms, has broadened its antibacterial spectrum without impairing its mildness, safety and other desirable bar soap characteristics. Properties of the new system, including in vitro and in vivo tests of its antibacterial activity, efficacy and safety, are discussed.

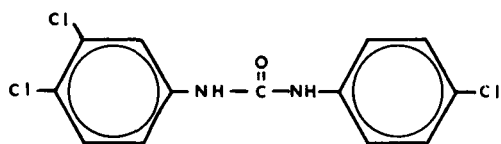
### INTRODUCTION

The use of antibacterial agents in toilet soaps has continued to grow to a point where, at present, more than 50% of the soap sold in the United States is categorized as deodorant or antibacterial soap, or both. It has been shown that the use of antibacterial soap contributes importantly to the reduction of gram-positive bacteria on the skin (1,2).

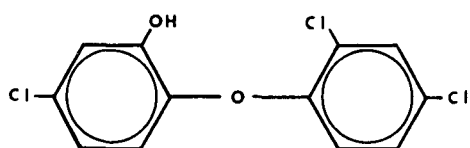
<sup>1</sup>Presented at the AOCS Meeting in New Orleans, La. April, 1970.



Hexachlorophene



3,4,4'-Trichlorocarbanilide  
(TCC, Triclocarban)



2-Hydroxy-2',4,4'-trichlorodiphenyl Ether

FIG. 1. Structural formulas of components of the L-52 system.

In addition, there is ample evidence that these products are clinically effective in reducing the incidence of simple cutaneous infections (3-6), erythrasma (7-9), in preventing diaper dermatitis (10), and in treating tropical ulcers (11). A particularly significant result was reported recently by MacKenzie (5) who compared an antibacterial soap containing 1.5% of a mixture of hexachlorophene and triclocarban during a six-month period at the U.S. Naval Academy to determine its ability to reduce the incidence of superficial cutaneous bacterial infections. He found that the incidence of these infections was decreased a statistically significant 44% in the group using antibacterial soap compared to an unmedicated bar.

Although the antibacterial systems which have been used in soap are very effective against a large number of gram-positive bacteria (12), they are relatively inactive against gram-negative organisms. The frequency of gram-negative infections in various hospitals has been reported to range from 1.5% to 6.4% (13-16). Hands must be considered among the vectors in transmission of gram-negative organisms, particularly *Salmonellae* (17-19).

In a previous paper, Jungermann (20) reported in detail on various techniques for screening soap bacteriostats. Using these techniques, a new germicide system was developed consisting of a mixture of 1% hexachlorophene, 1% 3,4,4'-trichlorocarbanilide (TCC, triclocarban) and 0.1% of a new antibacterial agent, 2-hydroxy-2',4,4'-trichlorodiphenyl ether (Irgasan DP 300, Geigy Chemical Co.) (21) (Fig. 1). This combination of ingredients, designated as L-52 (22,23), was found to possess antibacterial action against gram-positive and gram-negative microorganisms.

This paper is concerned with the properties of the new L-52 antibacterial system and reports the results of a series of in vitro and in vivo tests which were carried out to establish its antibacterial and deodorant effectiveness and safety. A soap containing a mixture (designated as AT-7) of 0.75% hexachlorophene and 0.75% TCC was used for comparison purposes in many of these experiments.

### EXPERIMENTAL PROCEDURES

#### Preparation of Test Bars

Neat soap pellets are made from 85% tallow fatty acids and 15% coconut fatty acids, neutralized with sodium hydroxide, and combined with the germicides and perfume. This mixture is passed three times through a three-roll mill. The resulting ribbons of soap are put through a Mazzoni duplex vacuum plodder and extruded as a long slug which is cut to proper lengths for stamping in a hand-operated bar soap press.

#### In Vitro Antibacterial Activity

*Agar Streak Dilution Technique.* In this test, described by Jungermann et al. (12), cultures of bacteria are transferred to nutrient medium containing known concentrations of soap; plates are incubated overnight at 37 C and the minimum concentrations of soap which will inhibit growth of the organisms are determined.

*Calfskin Disk Method.* In a modification of the method described by Vinson et al. (24), calfskin disks that have been steeped for 15 min in a soap solution are inbedded in agar and overlaid with agar seeded with the test culture, and

after overnight incubation at 37 C the zones of inhibition of growth are observed.

**Microbiological Availability Test.** According to the method of Taber et al. (25), circles of rehydrated calf-skin are placed in a specially designed device which prevents swelling and distortion, placed in contact with soap solution in various concentrations for various periods, inoculated with suitable dilutions of a culture of a test organism, and incubated in agar at 37 C for 48 hr. The number of colonies on each piece of calfskin are counted.

#### **In Vivo Antibacterial Activity**

**Hand Degerming.** The Cade (27) modification of the Price (26) method was used in our work; water is studied from the first, fourth and fifth basins and a brush is not used. Panelists use the test soap exclusively for washing, use no cosmetics, and are subjected to weekly test washes at which bacterial counts are done.

**Finger Imprinting.** In a modification of the finger imprint method described by Vinson et al. (24), panelists wash their hands for 3 min with the test soap, blot fingers dry with sterile paper towels, and imprint the index, middle and third fingers slightly on the surface of a seeded plate for several minutes. After incubation of plates, the number of colonies in the imprinted area is compared with that in a nonimprinted area.

**Hanging Drop Method.** A hanging drop slide is cut so that an area 2 x 2 cm contains one well. Nutrient agar containing 1:10,000 dilution of a 24 hr culture of *Escherichia coli* ATCC 4352 is placed in the well and allowed to harden. The slide is taped, well side down, to a palm that has been washed as in the finger imprint test. The slide remains taped to the palm for 7 hr and then is placed upright in a petri dish containing a sterile filter and incubated at 37 C overnight. The presence or absence of growth is observed under the microscope.

**Zones of Inhibition From Nail Clippings and Callus.** Subjects who have used a nonmedicated soap for all hand washing and bathing for a week before the test are assigned a test soap for all washing for one week. During four days the hands are washed under supervision three times for 3 min with the test soap. Immediately after the third supervised washing, the hands are rinsed and the fingertips scrubbed for a minute with a sterile brush and warm tap water. Then individual fingernail clippings and an excised callus are placed on agar seeded with *E. coli* ATCC 4352 and incubated overnight at 37 C. Zones of inhibition of growth are observed.

**Zones of Inhibition From Hair.** After daily shampooing for five days with test soap, rinsing for 30 sec, and drying, hair clipped from the frontal region of the scalp is wadded into a 1 cm diameter pad and incubated at 37 C in agar seeded as described above and zones of inhibition are observed.

#### **Deodorancy**

Fifty panelists wash both axillae with a nonmedicated soap for two weeks, then use test soaps, one for each axilla, under prescribed conditions, for one week. Evaluations by judges or by the panelists themselves are made daily of odor in the underarms or in the underarm areas of tee shirts. Results are analyzed statistically.

#### **Effect on Induced Infections.**

The method of Marples and Kligman (31) is employed to evaluate the effect of using soap with L-52 to retard clinical manifestations of infection when *Staphylococcus aureus* is allowed to grow under occlusion with Saran wrap on the ventral portion of the forearm of 11 volunteers. Under standard conditions the test site is washed three times daily for one week, soap containing L-52 and nonmedicated soap being used in a double-blind manner. The effect on existing

lesions and the appearance of new lesions are observed.

#### **Mildness**

**Test on Infants.** The method of Ellickson and Jungermann (33) was used to evaluate mildness. In an eight-week blind cross-over test, one group of babies is bathed exclusively with each of the test soaps for four weeks and then with the other soap for another four weeks. The condition of the skin in general and of the diaper area in particular is examined each week. Test soaps are identified by code, so the dermatologist who examines the babies is not aware of which soap is used. Nurses bathing the babies use the same soap for their own washing, and their hands are examined at the same times.

**Immersion Test.** The technique of Killian and Marsh (32) compares the irritating properties of soaps on men and women. Panelists sit between two tanks containing suspensions of the soaps and immerse one hand and forearm in each of two tanks for periods of 15 min at 3 hr intervals, twice the first day and three times on successive days. They alternate immersion for 1 min with air drying for 1/2 min, so that total immersion during any one period is for 10 min. Cosmetic and medicinal preparations are not used, but soaps of choice are used for other washing purposes. Hands and arms are examined each day for erythema, dryness and cracking. In this test, L-52 soap is compared with Ivory soap (Procter and Gamble Co).

## **RESULTS AND DISCUSSION**

#### **In Vitro Antibacterial Activity**

The minimum inhibitory concentrations (MIC) of soap containing the L-52 system (1% hexachlorophene, 1% TCC, 0.1% Irgasan DP 300) and a soap containing the AT-7 system (0.75% hexachlorophene, 0.75% TCC) was determined by the agar streak dilution technique (12) against 46 gram-positive and 34 gram-negative cultures. These included both common and uncommon pathogenic and nonpathogenic varieties. Results are shown in Tables I and II.

Against 42 of 46 gram-positive bacteria (Table I), the L-52 system was somewhat more effective, the differences being of the order of from 25% to 100%. Against almost all bacteria, both soaps had MICs below 10 ppm. Of special interest were differences in activity against strains of *S. aureus* and *Streptococcus faecalis* resistant to various antibiotics. Against all but one of these, the soap containing L-52 showed an increase in activity.

Against gram-negative bacteria (Table II), differences between the two soaps were of a much greater order. Reductions in MIC by as much as 3000% for L-52 were found compared with AT-7.

Comparisons of the effectiveness of the germicide systems with those of various antibiotics also are interesting. Such comparisons for AT-7 have been reported elsewhere (34,35). When the activity of soap containing L-52 against 11 gram-positive cultures was compared with that of eight antibiotics (Lincocin, Achromycin, Chloromycetin, Bicillin, Erythromycin, Penicillin G, Panalba and Vibramycin), only Bicillin and Penicillin G showed activity in a comparable range with that of L-52. Similar results were obtained with gram-negative organisms.

Table III shows the zones of inhibition of 14 gram-negative organisms by calfskin disks steeped in a solution of soap containing L-52 according to the method of Vinson et al. (24). Zones ranging in size from 0.5 mm to 8 mm were observed. Soap containing the AT-7 system did not produce zones.

Experiments in triplicate using the microbiological availability procedure to compare the effectiveness of a soap containing L-52 with that of a nonmedicated soap (Ivory) showed significantly lower ( $p < 0.05$ ) colony counts for

TABLE I

## In Vitro Effectiveness Against Gram-Positive Bacteria

Culture	Minimum Inhibitory Concentration, ppm of Total Product	
	L-52	AT-7
<b>Staphylococcus</b>		
<i>S. aureus</i> FDA 209	4	5
<i>S. epidermidis</i> ATCC 155	4	6
PS 42D	2	3
PS 187	2-3	3
SA 9 Smith (diffuse)	2	3
Wood 46	3	5
Cowan I NCTC 8530	4	5
Cowan II NCTC 8531	3	5
DA 3008-7	4	6
DA 2871-7	5	5
DA 2620-7	4	7
DA 2614-7	6	7
DA 2970-7	4	5
DA 2969-7	4	5
W-2	3	5-6
W-11	3	4
W-14	3	5
W-5	3	4
<i>S. epidermidis</i> W-10	3	4
<i>S. aureus</i> ATCC 11631 (penicillin resistant)	5	7
<i>S. aureus</i> ATCC 11632 (penicillin resistant)	6	6
<i>S. aureus</i> ATCC 12715 (Tetracycline, chlorotetracycline and oxytetracycline resistant)	10	20
<i>S. aureus</i> ATCC 14829 (neomycin resistant)	5	7
<i>S. aureus</i> ATCC 11301 (penicillin resistant)	2	3
<i>S. aureus</i> ATCC 11302 (penicillin resistant)	3	5
<i>S. aureus</i> ATCC 11371	2	4
<i>S. aureus</i> 55/71	2	3
<i>S. aureus</i> 3A/3C	2	3
<i>S. aureus</i> 54/77	2	3
<i>S. aureus</i> 52A/79	2	3
<i>S. aureus</i> 80/81	3	5
<b>Streptococcus</b>		
Group B, B-5	7	9
Group A, SS 510	3	4
Alpha Group D, DS1455-65	8	20
Group G, DS1426-65	3	4
Group B, B-1	7	20
Group A, GS 208-4	3	5
<i>S. pyogenes</i> B624	7	8
<i>S. faecalis</i> ATCC 14506 (neomycin resistant)	20	30
<i>S. faecalis</i> ATCC 14507 (kanamycin resistant)	20	20
<b>Corynebacterium</b>		
<i>C. hoagii</i> F-17	7	9
<i>C. pseudodiphtheriticum</i> ATCC 10700	9	30
<b>Bacillus</b>		
<i>B. cereus</i> ATCC 9592 (penicillin resistant)	6	7
<i>B. subtilis</i> ATCC 6460	6	7
<b>Miscellaneous</b>		
<i>Sarcina lutea</i> ATCC 9341	3	5
<i>Brevibacterium ammoniagenes</i> ATCC 6871	3	3

TABLE II

## In Vitro Effectiveness Against Gram-Negative Bacteria

Culture	Minimum inhibitory concentration, ppm of total product	
	L-52	AT-7
<b>Salmonella</b>		
<i>S. oranienburg</i>	250	4,000
<i>S. typhi</i>	300	10,000
<i>S. newport</i>	500	9,000
<i>S. nottingham</i>	300	9,000
<i>S. typhimurium</i>	500	10,000
<i>S. typhi</i> 13592	200	6,000
<i>S. bredeney</i>	400	10,000
<i>S. java</i>	500	10,000
<i>S. enteritidis</i>	300	10,000
<i>S. urbana</i>	300	10,000
<i>S. infantis</i>	500	10,000
<i>S. newington</i>	300	10,000
<i>S. typhosa</i> ATCC 6539	100	6,000
<b>Shigella</b>		
<i>S. flexneri</i>	100	7,000
<i>S. sonnei</i>	200	7,000
<i>S. dysenteriae</i>	100	3,000
<b>Proteus</b>		
<i>P. mirabilis</i>	200	1,000
<i>A. faecalis</i>	300	500
<b>Escherichia</b>		
<i>E. coli</i> 086a: B7: H8: 4924-66	200	5,000
<i>E. coli</i> 026: B6: H11: 2139-67	300	2,000
<i>E. coli</i> 055: B5: H7: 3934-67	200	5,000
<i>E. coli</i> 0119: B14: H6: 2607-67	200	5,000
<i>E. coli</i> 0128a: 128b: B12: H2: 5145-66	300	5,000
<i>E. coli</i> 0125a: 125c: B15: H30: 3178-66	300	5,000
<i>E. coli</i> 0126: B16: H27: 489-67	300	5,000
<i>E. coli</i> 0127a: B8: H21: 6129-66	200	5,000
<i>E. coli</i> 0116a: 11b: B4: H12: 2278-67	200	5,000
<i>E. coli</i> ATCC 11229	200	6,000
<i>E. coli</i> ATCC 4352	60	2,000
<b>Aerobacter</b>		
<i>A. aerogenes</i> ATCC 12409	200	8,000
<b>Klebsiella</b>		
<i>K. pneumoniae</i> ATCC 9590	200	8,000
<i>K. pneumoniae</i> ATCC 9591 (penicillin resistant)	200	8,000
<b>Herellea</b>		
<i>Herellea</i> sp. ATCC 11959	200	8,000
<b>Arizona</b>		
<i>A. arizonae</i> ATCC 12323	200	8,000

variance this difference was found to be significant ( $p < 0.05$ ).

## In Vivo Antibacterial Activity

**Hand Degerming.** A basic requirement of an antibacterial soap is that it diminish the number of bacteria in the deeper layers of the skin. Price (26) distinguished between the transient microorganisms on the outermost part of the skin, which are simply transferred from the environment to the skin and can be removed with an ordinary washing, and the resident bacteria.

Table V shows the difference between soap containing L-52 and nonmedicated soap when the Cade (27) modification of the Price test was used to compare the bacterial counts in the fifth wash water before and after 12 days' washing with the test soaps. The geometric mean difference was significant at  $p < 0.01$  (t test). Reductions of up to 96% were achieved.

**Finger Imprinting.** Because few gram-negative bacteria normally are present in the skin, a finger imprint method was used to compare the effects of soap containing L-52 and nonmedicated soap against such organisms. After a week's use of nonmedicated soap for all bathing and

each of 10 gram-positive cultures for L-52 than for nonmedicated soap (Table IV).

A modified microbiological availability procedure simulating the effect in actual practice of several washings was used for the evaluation of the gram-negative organism, *E. coli* ATCC 11229. In this test, the effect on the organism of an 8% solution of L-52 soap was determined after 10 min contact. The average of three determinations of bacteria surviving exposure to nonmedicated soap was 3604, compared with 2624 surviving exposure to L-52. By analysis of

TABLE III

Zones of Inhibition Against Gram-Negative Bacteria<sup>a</sup>

Culture	Zone dimension, mm <sup>b</sup>
<i>Escherichia coli</i> ATCC 4352	8
<i>E. coli</i> ATCC 11229	1.7
<i>E. coli</i> 0116a: 11b: B4: H12:2278-67	2.5
<i>E. coli</i> 0127a: B8:H21:6129-66	1.7
<i>E. coli</i> 0126:B16:H27:489-67	1.8
<i>Salmonella newington</i>	2.5
<i>S. urbana</i>	0.5
<i>S. infantis</i>	1.2
<i>S. enteritidis</i>	1.3
<i>S. typhimurium</i>	0.5
<i>S. pullorum</i>	4
<i>S. typhosa</i> ATCC 6539	3.7
<i>Klebsiella pneumoniae</i> ATCC 9590	3
<i>Arizona arizonae</i> ATCC 12323	1.2

<sup>a</sup>Vinson Method (21).  
<sup>b</sup>Average of three determinations.

washing, 21 subjects used soap containing L-52 and 19 used nonmedicated soap from Monday morning through Thursday afternoon and then pressed their fingertips in agar seeded with *Salmonella newington* and five cultures of *E. coli*. Zones of inhibition of all six organisms were observed when L-52 soap was used.

**Hanging Drop Method.** Slides inoculated with *E. coli* ATCC 4352 and taped to hands washed with soap containing L-52 showed no growth of bacteria after incubation, but those taped to hands washed in nonmedicated soap showed heavy growth (Figs. 2,3). This is an especially dramatic example of the residual effect of L-52; it demonstrates the inhibition of gram-negative bacteria in an environment (temperature, moisture, nutriment) which is in fact ideal for their growth. The absence of growth is due to the presence of residual bacteriostat on skin which can diffuse into the agar in an amount sufficient to prevent bacterial growth.

**Zones of Inhibition by Nail Clippings, Callus and Hair.** After repeated washings with soap containing L-52, nail clippings, excised callus, and hair incubated at 37 C overnight on plates seeded with *E. coli* ATCC 4352, all showed zones of inhibition of bacterial growth. These results are consistent with data from calfskin substantivity testing, finger imprinting, hand degerming evaluation and microbiological availability, and inhibition of bacteria on occluded skin. Taken together, these findings affirm the substantivity to protein (skin, hair, nails) of the antibacterial ingredients in L-52 in amounts sufficient to be inhibitory to a variety of bacterial cultures.

TABLE IV

Average Viable Cell Count After Exposure of Staphylococcal Strains to L-52 and Nonmedicated Soap<sup>a</sup>

	Nonmedicated soap	L-52 soap
Cowan I, NCTC 8530	2,101	828
Cowan II, NCTC 8531	2,554	1,365
DA 3008-7	2,534	1,857
DA 2620-7	4,801	385
Wood 46	4,247	111
SA 9 Smith, (diffuse)	4,242	68
PS 42 D	4,672	337
DA 2614-7	4,812	3,423
ATCC 11631 <sup>b</sup>	3,012	1,997
ATCC 6538	5,315	3,965

<sup>a</sup>Tests in triplicate.  
<sup>b</sup>This culture is penicillin resistant.

**Deodorancy**

Much of the popularity of antibacterial soaps has been based on their deodorant action, which results from inhibition of the growth of odor-producing bacteria. Two gram-positive microorganisms, *Staphylococcus epidermidis* and *Corynebacterium diphtheriticum*, isolated from human axillae, have been reported (28) to elicit typical body odor from sterile sweat to a greater extent than other organisms recovered from the axillae. Dravnieks et al. (29,30) reported in detail on the effects of a soap containing AT-7 on the axillary bacterial population, intensity of primary and secondary odor, and water production, and Jungermann (20) compared the deodorant effectiveness of this soap with other deodorant soaps.

When compared to a deodorant soap containing a mixture of 0.75% hexachlorophene and 0.75% triclocarban, L-52 soap was found to provide longer lasting protection after all washing of the axillae had ceased. Tee shirt ratings at the end of the first post-test day showed a preference for soap containing L-52, (one-sided p value = 0.06 by the sign test). The difference at the end of the second post-test day also had about a 6% one-sided probability of occurrence by chance. By the end of the third day the difference had disappeared.

**Effect on Induced Infections**

Using the method of Marples and Kligman (31), nine of 11 volunteers completed the retardation part of the study. With inocula of from 10<sup>7</sup> to 10<sup>9</sup> cells in 0.025 ml of saline solution, there was a marked reduction in incidence and

TABLE V

Cade Handwashing Test Using L-52 at Basin Number 5

Subpanel	Subject	Log Bacterial Count		Difference <sup>a</sup> D
		L-52 day no. 12	Control day no. 1	
1	1	4.58	6.39	- 1.81
	2	5.21	6.52	- 1.31
	3	5.62	6.73	- 1.11
	4	5.33	6.14	- 0.81
	5	5.58	5.93	- 0.35
	6	5.32	6.27	- 0.95
2	7	5.73	6.38	- 0.65
	8	5.51	6.51	- 1.00
	9	5.61	6.57	- 0.96
	10	5.47	6.39	- 0.92
	11	4.38	6.51	- 2.13
	12	5.53	6.41	- 0.88
Sums				-12.88

<sup>a</sup>Geometric mean difference =  $\bar{d}$  = -1.07; standard error of mean difference =  $\bar{d}$  = 0.140.

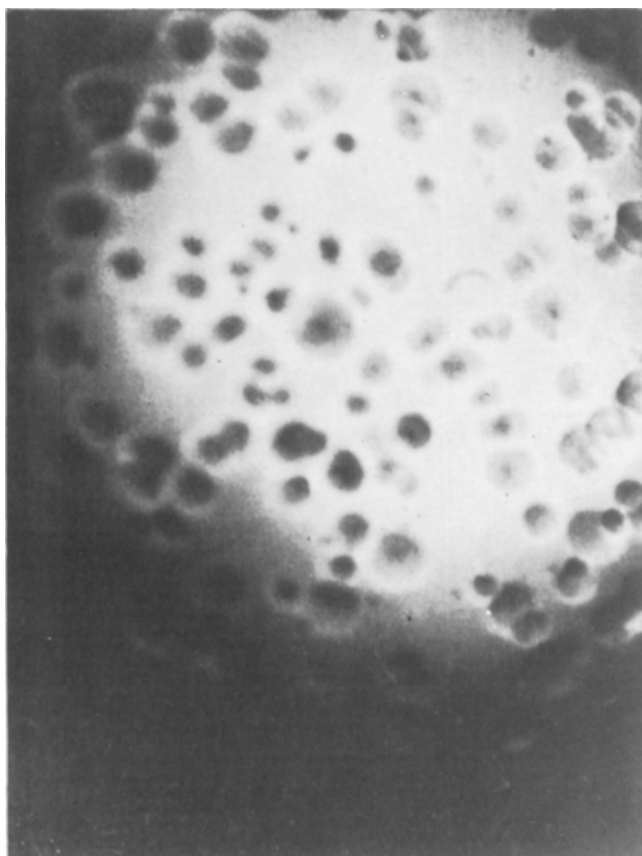


FIG. 2. Growth of *E. coli* ATCC 4352, hands washed with Ivory. Magnified 100 x, reduced approximately 60%.

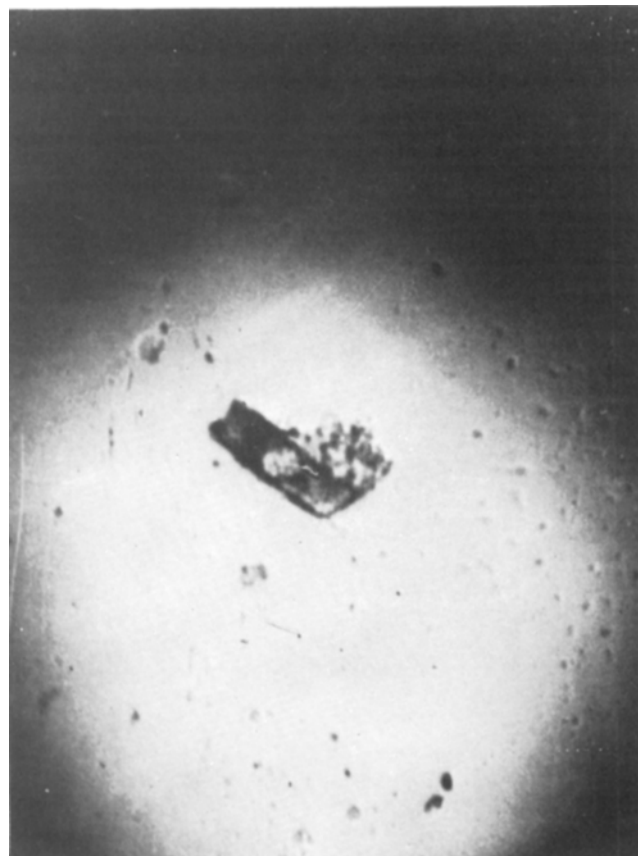


FIG. 3. Absence of growth of *E. coli* ATCC 4352, hands washed with L-52 soap. Magnified 100 x, reduced approximately 60%. The dark object is an artifact.

severity of infection at sites washed with soap containing L-52 (36). When infections induced on arms washed with nonmedicated soap were treated with solutions of soap containing L-52 or nonmedicated soaps, the L-52 soap was more efficacious than nonmedicated soap in preventing the appearance of new lesions during the period of treatment and in ameliorating the clinical manifestations of infection.

These results provide a rationale for the observation that use of antibacterial soaps helps to prevent simple infections of the skin (3-9). Treatment with such soap causes an antibacterial agent to be deposited on the skin which can inhibit growth of pathogenic bacteria.

#### Mildness

The sensitive skin of infants is particularly suitable for a test of the mildness of soap. Previously, the use of soap containing AT-7 for bathing infants had been reported (10) to reduce the incidence and severity of diaper rash. In another study (33) soap with AT-7 was found to be as mild

TABLE VI  
Mildness by Arm Immersion

Reaction	Incidence of reaction, %	
	L-52	Nonmedicated soap
Erythema		
Mild	42	37
Moderate	34	24
Severe	6	21
Drying		
Mild	72	70
Moderate	20	21
Severe	1	1
Cracking		
Mild	1	5

as a nonmedicated soap (Ivory) when it was used for routine washing and bathing of infants.

In the present study, the response of infants' skin to soap containing L-52 was compared with that of a nonmedicated soap (Ivory). The study involved 151 infants between 1 and 16 months old. One hundred and forty infants remained in the hospital more than a week after the study was undertaken; of these, 51 completed four weeks on each soap and the others were bathed with each soap for varying periods. There was no evidence of primary irritation or allergic contact dermatitis from use of either soap containing L-52 or Ivory.

When using the Killian and Marsh immersion technique (32) in comparing soap with L-52 system vs. nonmedicated soap (Ivory), the only significant difference ( $p = 0.01$ ) was in erythema. In this case the L-52 soap was favored. Table VI shows the incidence of erythema, dryness and cracking for the entire test period.

#### Safety

The safety of L-52 was tested to an extent that probably is unique in the evaluation of antibacterial soaps. A partial list of the tests carried out are the following:

##### 1. Animal Toxicology of L-52:

- Acute Oral Toxicity (rats)
- Acute Oral Toxicity (dogs)
- Eye Irritation Study (rabbits)
- Skin Irritation Study (rabbits)
- Skin Sensitization Study (guinea pigs)
- 20 Day Subacute Dermal Study (rabbits)
- 90 Day Subacute Dermal Study (rabbits)
- Acute Dermal Toxicity (rabbits)

##### 2. Animal Toxicology of Soap Containing L-52:

- 90 Day Subacute Percutaneous Study (rabbits)
- Acute Oral Toxicity (rats)
- Acute Oral Toxicity (dogs)

Acute Dermal Toxicity (rabbits)  
 21-Day Subacute Dermal Toxicity (rabbits)  
 Subacute Dermal Toxicity Study  
 (neonatal beagle pups)  
 Irritating and Toxic Effects of a Two Per Cent  
 Solution of L-52 Soap Used as an Enema in Beagle Pups  
 Skin Irritation (rabbits)  
 Eye Irritation (rabbits)  
 3. Human Testing:  
 Human Systemic Toxicity  
 Photosensitization  
 Draize Type Allergic Sensitization  
 Primary Irritation (Finkelstein Method)  
 Skin Irritating Properties  
 Comparative Mildness in Babies  
 Soap Use at State Prison  
 Soap Use at Detention Home for Juveniles  
 Market Test of Soap  
 Deodorant Properties  
 Percutaneous Penetration of Components of L-52  
 Extensive Consumer Testing

The testing included detailed toxicological studies on the germicide system per se, as well as in soap. Extensive human testing was also carried out by medical investigators. In all, approximately 3000 human subjects, infants, youths and adults, used the soap for periods up to six months under medical supervision. Some information on infant use has been described in the section on mildness. Not a single case of contact sensitization or photosensitization was reported during any of these tests. Details of these safety evaluations will be reported elsewhere.

#### Clinical Studies

As stated earlier, clinical experience with soap containing AT-7 has shown it to be effective in preventing simple skin infections (4,5), in moderating some of the symptoms associated with diaper dermatitis (R.R. Marples and A.M. Kligman, personal communication), and in treating erythrasma of the toe webs (9). There is also some evidence that the product may assist the healing of tropical ulcers (11).

Studies of a similar nature have shown that soap containing L-52 also is of benefit in these conditions. An unusual property of the new soap is its effect on the severity of tinea pedis. Publication of these findings will appear elsewhere.

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