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**The Effect of Shower/Bath Frequency on the Health
and Operational Effectiveness of Soldiers in a Field
Setting: Recommendation of Showering
Frequencies for Reducing Performance-Degrading
Nonsystemic Microbial Skin Infections**

**L. C. Hall
J. I. Daniels
R. Aly
H. I. Maibach
S. A. Schaub
L. E. Becker**

September 30, 1991



**Lawrence
Livermore
National
Laboratory**

**Supported by
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L. C. Hall
J. I. Daniels
R. Aly*
H. I. Maibach*
S. A. Schaub†
L. E. Becker‡

Environmental Sciences Division
Lawrence Livermore National Laboratory
University of California
P.O. Box 5507
Livermore, CA 94550-0617

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Contracting Officer's Representative: Dr. Stephen A. Schaub

Principal Investigators at Lawrence Livermore National Laboratory:
L. C. Hall and J. I. Daniels

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* Department of Dermatology, University of California School of Medicine, San Francisco, CA 94143.

† U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, MD 21702-5010.

‡ Dermatology Consultant to the Office of the U.S. Army Surgeon General, Brooke Army Medical Center,
Fort Sam Houston, TX 78234-6200.

Preface

For soldiers in a combat setting, the ability to shower, bathe, or otherwise maintain personal hygiene may be an important factor in the maintenance of health and morale. Currently, the U.S. Army makes washing facilities available to soldiers in the field on a weekly basis (U.S. Army, 1982a; and 1990). This bathing regimen was established to disrupt the life-cycle of the body louse (Busvine, 1985; and U.S. Army, 1982a, and 1990), but there is no definitive evidence that it is adequate to minimize or prevent microbial diseases of the skin. For soldiers in the field, morbidity from microbial skin infections, particularly in hot, humid environments, has caused significant losses of manpower; U.S. Army after-action reports, as well as anecdotal descriptions from the field, indicate that showering once a week may not be an optimal regimen for the maintenance of personal hygiene, especially with respect to microbial diseases of the skin (U. S. Army, 1990; and Troychock, 1991).

Recognition that the current policy on shower frequency may be inadequate was one of several factors that led the U.S. Army Medical Research and Development Command to fund a project to determine an optimum showering frequency for combat troops. Planning and logistical considerations, and the need to determine the quantity of water required for showering also contributed to the decision (U.S. Army, 1982b, and 1990). The U.S. Army has recognized that nonmedical issues such as troop morale also are important factors in establishing an optimum showering frequency (U.S. Army, 1982a). It was beyond the scope of the present effort, however, to evaluate the impact of showering frequency on morale or other nonmedical factors.

The principal goal of this project was to determine an optimum showering frequency for soldiers in the field based on an evaluation of published literature on the relationship between showering frequency, microorganisms that cause skin disease, and other factors that cause or contribute to diseases of the skin, such as climate and the microclimate produced by clothing. We also examined the efficacy of water, unmedicated and antimicrobial soaps, and chlorhexidine antiseptic in the removal of microorganisms to determine if the use of a specific cleansing product or substance could minimize the incidence and/or severity of dermal disease under field conditions.

Over the course of the project, we obtained information from a number of sources; discussions with physicians and scientists active in dermatology, microbiology, and military medicine provided us with important perspectives and numerous scientific citations. Searches of the computerized data bases, Medline, Biosis, Chemical Abstracts, National Technical Information Service, the

Aerospace Data Base, Nuclear Science Abstracts, DOE Energy Abstracts, EM Base, and the Defense Technical Information Center (DTIC), also yielded many important references. During the 1960's and 1970's, the Letterman Army Institute of Research (LAIR) at the Presidio in San Francisco, California, was the site of an active dermatology research unit. We reviewed a number of Annual Research Progress Reports from LAIR (1972, 1973, 1976, 1977, and 1978) that contained citations for publications resulting from research conducted at that facility. We also reviewed the collected papers of the late Dr. Marion Sulzberger (available at the Library of the University of California, San Francisco), who was head of the Dermatology Research Division at LAIR from 1964 to 1970. In addition to these sources of information, Colonel John Troychock (U.S. Army Reserves) was asked by the U.S. Army to acquire and examine hard-to-obtain archived military documents, after-action reports, and official bulletins and correspondence concerning the relationship between showering, bathing, and prevention of skin disease. We evaluated the contents of the written overview of this material that was submitted by Colonel Troychock (1991) to Dr. Stephen A. Schaub at the Health Effects Research Division of the U.S. Army Biomedical Research and Development Laboratory at Fort Detrick in Frederick, MD.

Furthermore, during the 1960's and early 1970's, the U.S. Army Medical Research and Development Command (MRDC), sponsored an array of field research projects to identify the biological and physical factors that cause and/or contribute to the development of skin diseases in the tropics. Some of this research was performed at the University of Miami in Florida. According to the results of our literature search, the majority of the U.S. Army MRDC funded research on this subject was never published in peer-reviewed scientific literature, and only a limited amount of this research appears to be available in U.S. Army reports. However, we did obtain a few of the relevant U.S. Army annual reports and scientific articles that were prepared at the University of Miami (discussed below).

Researchers from the University of Miami had a long and productive working relationship with the U.S. Army MRDC. In 1962, a team of scientists and physicians from the University of Miami spent three months in Panama with U.S. troops participating in Operation Swampfox II. The focus of their research was to identify the causative organisms of skin infections among a military population in the field, to determine the prevalence of each type of infection, and to identify the role of environmental factors in the etiology of skin disease in the tropics (Taplin *et al.*, 1965; and Taplin *et al.*, 1967). The causative organisms identified by these researchers are among those listed in Table 2-1 (see Chapter 2) of this report; the role of

environmental factors in the etiology of skin disease also is discussed in Chapter 2 in the section titled "Factors that Promote Microbial Infections of the Skin."

During the Vietnam war, University of Miami scientists performed a series of field epidemiology studies that were funded by the U.S. Army and were similar in focus to those completed in Panama during Operation Swampfox II. Although our search of the DTIC data base in 1990 did not result in any citations of U.S. Army reports that covered that research, much of that work was described by Alien (1989) in the text *Skin Diseases in Vietnam*—a reference work that was of great value to us and which we cite frequently in this report. Moreover, the field-research in Vietnam provided material for a number of journal articles, many of which also are discussed in this report.

Following the Vietnam war, the University of Miami continued to receive funding from the U.S. Army MRDC to study the etiology of bacterial and fungal skin diseases in the tropics. We examined the contents of the two annual progress reports (Taplin, 1975: for the period June 1, 1973, to May 31, 1974; and Taplin, 1976: for the period June 1, 1974 to December 31, 1975) and the one final report (Taplin, 1978: for the period June 1, 1971 to May 31, 1976) describing this research, which were found as a result of our literature search. These reports contain descriptions of a clinical trial of an antimicrobial soap and a clinical trial of the antiseptic chlorhexidine (reviewed in Appendix A), as well as descriptions of infections in survivors of a plane crash in the Everglades, the role of water temperature in the development of "immersion foot syndrome," an outbreak of furunculosis (i.e., boils) in Florida. Also presented in these reports are evaluations of various medications for the treatment of ringworm, and results of surveys of skin disease among units of the Colombian Army, and of skin infections among children in Costa Rica. Although the research funded by the U.S. Army MRDC that is described in the reports and articles that we obtained yielded a great deal of valuable information, none of this information specifically addresses showering or bathing frequency and its influence (or lack of influence) on the development of microbial diseases of the skin.

The first chapter of this report contains introductory comments and a review of the historical significance of microbial infections of the skin among military personnel in the field. In Chapter 2, we evaluate data on microbial diseases of the skin and discuss the factors that promote development of these diseases. Chapter 3 and Appendix A contain a discussion of the efficacy of water, soap, and chlorhexidine in removing microorganisms from the skin. Appendix B contains a list of microorganisms that cause nonsystemic diseases of the skin (including available references). Chapter 4 presents the equations used to calculate an optimum showering

frequency, discusses the predicted optimum showering frequencies, and describes the assumptions and limitations of our approach. In Chapter 5, we summarize our conclusions and make recommendations for future research.

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The Effect of Shower/Bath Frequency on the Health and Operational Effectiveness of Soldiers in a Field Setting: Recommendation of Showering Frequencies for Reducing Performance-Degrading Nonsystemic Microbial Skin Infections

Abstract

Historically, military personnel deployed in the field, particularly in hot, humid environments, have suffered disabling microbial infections of the skin severe enough to contribute to significant reductions in combat-troop strength. Currently, the U.S. Army makes facilities available to field personnel for showering on a weekly basis to prevent infestations of the body louse and the subsequent spread of louse-borne disease. However, a weekly showering frequency has never been evaluated for its efficacy in preventing microbial infections of the skin—a significant cause of man-days lost from combat in modern-day military conflicts. Consequently, field showers may be more important for maintaining combat effectiveness of military personnel than previously thought; however, providing such facilities requires tremendous logistical support. Therefore, we developed shower frequencies for troops in field environments that should minimize or prevent microbial skin infections. According to our calculations, the optimum showering frequency can range from as often as four times per day to as little as once every seven days, depending on skin integrity, environmental conditions, and cleansing agent. We also reviewed the scientific and regulatory information concerning the efficacy and safety of skin-cleansing products; the antimicrobial and antiseptic compounds, triclocarban and chlorhexidine, may be the most suitable for routine use by U.S. military personnel.

The Effect of Shower/Bath Frequency on the Health and Operational Effectiveness of Soldiers in a Field Setting: Recommendation of Showering Frequencies for Reducing Performance-Degrading Nonsystemic Microbial Skin Infections

Executive Summary

Historically, military personnel deployed in field environments have suffered disabling microbial infections of the skin severe enough to contribute to significant reductions in combat-troop strength. To prevent infestations of the body louse and the subsequent spread of louse-borne disease, the U.S. Army makes facilities available to military personnel in the field for showering on a weekly basis. However, a weekly showering frequency has never been evaluated rigorously for its efficacy in preventing microbial infections of the skin—a significant cause of man-days lost from combat in modern-day military conflicts. Consequently, field showers may be even more important for maintaining combat effectiveness of military personnel than previously thought; however, providing such field facilities requires tremendous logistical support. Accordingly, military planners must carefully consider the appropriate shower frequency necessary to minimize the incidence and/or severity of dermal disease under field conditions. For these reasons, the U.S. Army Medical Research and Development Command needed to determine a shower frequency for troops deployed in field environments that should minimize or prevent microbial skin infections.

L. Hali and J. Daniels at the University of California, Lawrence Livermore National Laboratory (LLNL), and R. Aly and H. Maibach at the University of California, San Francisco (UCSF), were funded by the U.S. Army Medical Research and Development Command to develop recommendations of showering frequency for military personnel in the field. These recommendations were developed in collaboration with S. Schaub at the U.S. Army Biomedical Research and Development Laboratory (USABRDL) at Fort Detrick in Frederick, MD, and Col. L. Becker at Brooke Army Medical Center at Fort Sam Houston, TX. This Executive Summary briefly discusses the recommendations and relevant supporting information.

Overview

The principal goal of this project was to determine an optimum showering frequency for military personnel in the field based on an evaluation of published literature on the relationship between showering frequency, microorganisms that cause skin disease, and other factors that cause or contribute to diseases of the skin, such as climate and the microclimate produced by clothing. First, we examined how microbial populations on the skin change with time under varying environmental and microenvironmental conditions (e.g., hydration and occlusion). Additionally, we used published data on experimental induction of microbial skin infections to determine the average population level of selected (i.e., indicator) microorganisms that can initiate disease. Next, we evaluated data on the efficacy with which water alone or water in combination with plain soap, antimicrobial soap, or chlorhexidine antiseptic removes microorganisms from the skin surface. Finally, on the basis of all these data, we were able to (1) determine the population dynamics of pathogenic indicator microorganisms on the skin under different environmental conditions and cleansing procedures and (2) predict when and under what circumstances these populations reach levels that could produce infection on both intact skin and skin compromised by minor wounds (e.g., mechanical trauma, abrasion, and insect bites).

The first chapter of this report contains introductory comments and a review of the historical significance of microbial infections of the skin among military personnel in the field. In Chapter 2, we evaluate data on microbial diseases of the skin and discuss the factors that promote development of these diseases. Chapter 3 and Appendix A contain a discussion of the efficacy of water, soap, and chlorhexidine in removing microorganisms from the skin. Appendix B contains a list of microorganisms that cause nonsystemic diseases of the skin. Chapter 4 describes the equations used to calculate an optimum showering frequency, discusses the predicted optimum showering frequencies, and the assumptions and limitations of our approach. In Chapter 5, we summarize our conclusions and make recommendations for future research.

Microbial Infections of the Skin

Microbial infections of the skin caused significant decrements of combat troop strength in WW II and Vietnam (see Chapter 1). Data from these two conflicts consistently implicate a relatively small number of microorganisms (i.e., the fungi *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Trichophyton rubrum*, and *Candida albicans*, and the bacteria *Staphylococcus aureus* and *Streptococcus*

pyogenes) as causing the vast majority of skin disease. These and the other microorganisms that can cause nonsystemic diseases of the skin and therefore may be of significance to U.S. Army military personnel and planners are listed in Table 2-1 in Chapter 2.

Microbial infections of the skin develop when a combination of factors exists; the presence of a particular pathogenic microorganism is only one factor. The integrity of the skin must also be altered—by abrasion or wounding, or by occlusion and hydration (which can promote the growth of pathogenic microorganisms and lead to an accumulation of cytolytic microbial toxins). Furthermore, prolonged contact of the skin with sweat, urine, feces, and/or soil may contribute to microbial infections of the skin in military personnel in the field. In fact, experimental data confirm empirical reports from the field (e.g., Vietnam, Colombia, Malaya, the southern United States, and Panama) that a direct correlation between heat, humidity, skin trauma, and skin disease probably exists.

We obtained the data on the experimental induction of infection for five pathogenic microorganisms: *Trichophyton mentagrophytes*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. However, there were sufficient data only for *Staphylococcus aureus* and *Candida albicans* to model the development of microbial infections of the skin, which was essential for the calculation of an optimum showering frequency.

Effectiveness of Cleansing in the Prevention of Microbial Infections of the Skin

To calculate an optimum showering frequency for military personnel in a field setting, we analyzed data specifically identifying the efficiency of a single shower with water, or water in combination with unmedicated soaps, medicated soaps, or chlorhexidine antiseptic for reducing populations of microorganisms on human skin. Appendix A provides the historical perspective for this discussion and presents a comprehensive review of the scientific and regulatory information concerning the efficacy and safety of cleansing products with antimicrobial activity that may be suitable for routine use by U.S. military personnel in field environments. We determined that a single shower with chlorhexidine can remove approximately 94% of skin-surface microorganisms; a single shower with an unmedicated bar soap can remove approximately 84% of skin-surface microorganisms; and a single shower with water alone can remove approximately 77% of skin-surface microorganisms.

Although specific data for medicated bar soap were not available, we concluded that its efficiency for removing skin-surface microorganisms should range from 84% to 94%.

We used these cleansing efficiencies in conjunction with information on the population dynamics of the indicator microorganisms, *Staphylococcus aureus* and *Candida albicans*, and data on the effect of occlusion and wounding on the growth of skin microorganisms to calculate an interval between showers that should prevent pathogenic microorganisms from reaching populations on the skin capable of initiating infection. In the absence of data to the contrary, we assumed that the efficacy of cleansing with water, water with an unmedicated soap, or water with chlorhexidine on populations of indicator microorganisms will be the same for all potentially pathogenic microorganisms.

Recommendations for Showering Intervals

We developed mathematical equations describing the conceptual model of microbial population dynamics on the skin. (These are presented in Chapter 4.) On the basis of the application of these equations, we calculated showering intervals that should prevent or minimize the severity of microbial infections of the skin. On the basis of the showering intervals that were calculated (see Tables 4-2 through 4-4) we make the following practical recommendations:

- To prevent a microbial infection from developing on occluded but relatively healthy human skin (i.e., intact skin) the interval between showers for military populations deployed in tropical environments (or in environments where periodically a combination of temperature, humidity, and precipitation may approximate that of the tropics) should not exceed (1) 24 h (exactly 1 d), if chlorhexidine antiseptic is used with water (or slightly less than 24 h, if a medicated soap is used with water); (2) 19 h (for practical purposes, 1 d), if unmedicated soap is used with water; or (3) 15 h (about 0.5 d), if only water is used;

- To prevent a microbial infection from developing on wounded, abraded, or otherwise damaged human skin that has been occluded, the interval between showers for military populations deployed in tropical environments (or in environments where periodically a combination of temperature, humidity, and precipitation may approximate that of the tropics) should not exceed (1) approximately 12 h (about 0.5 d), if chlorhexidine antiseptic is used with water (or slightly less than 12 h, if a medicated soap is used with water); (2) about 7.5 h (approximately 0.3 d), if unmedicated soap is used with water; or (3) 6 h (exactly 0.25 d), if only water is used; and
- The maximum interval between showers should never exceed 7 d, whether or not water is used alone or with a cleansing agent; a 7-d interval should prevent infestations of the body louse and subsequent spread of louse-borne disease, as well as provide a positive effect on morale.

The showering intervals identified above are based on preventing skin infection in military personnel, determined either from the results of limited experiments involving inoculation of the skin with microorganisms followed by complete occlusion with a relatively impermeable plastic wrap (e.g., Saran Wrap), or are based on the disruption of the life cycle of the body louse. Until more complete information is available concerning relationships between the relative health of the skin and (1) environmental conditions (e.g., temperate, arctic, or desert climates that are geographical and/or seasonal), (2) fabric coverings with occlusive properties different from Saran Wrap, and (3) the efficacy of cleansing agents, a more accurate range of showering intervals that is applicable to each specific environment that may be encountered by U.S. military personnel cannot be provided.

Conclusions and Research Recommendations

We have used the limited data on the development of microbial infections of the skin and on the prevention of such infections by showering with water or water and a cleansing agent (available in the peer reviewed scientific literature) to calculate showering frequencies that should prevent nonsystemic microbial infections of the

skin in military personnel. Although there is uncertainty associated with our recommendations, there is a strong element of conservatism incorporated into each one. Our analyses indicate that adherence to a specific showering frequency can be an effective mechanism for preventing skin disease, but that the interval of time that should be allowed to elapse between showers most likely will depend on the cleansing agent that is used. For example, microbial populations are more likely to recover in size rapidly between showers with water alone or water with medicated or unmedicated soap than between showers with water and chlorhexidine antiseptic. Consequently, if water alone or water and medicated or unmedicated soap are used in showering, then it is important that the recommended showering frequency be strictly observed. [Provision of bathing facilities (e.g., bath tubs) is unlikely in a military setting and data provided by Byrne *et al.* (1990) indicate that bathing is not as efficient as showering in reducing populations of microorganisms on the skin. Accordingly, regular bathing was not considered a practical option for military personnel deployed in a field environment.]

We also conclude that under conditions where skin has been wounded and also is occluded, an interval between showers of much less than one day may need to be adopted, no matter what cleansing agent is used. For example, under such conditions, showering intervals ranging from a maximum of 0.5 d to a minimum of 0.25 d are estimated for showering with water and chlorhexidine or for showering with water alone. Furthermore, there are no data available to calculate an interval between showers for individuals under conditions that are not conducive to optimal microbial growth. Consequently, under such conditions (e.g., nonocclusive clothing worn intermittently in a temperate climate), we recommend that the U.S. Army continue to provide showers at least once every 7 d. The basis for this recommendation is that showering every 7 d will disrupt the life cycle of the body louse, which can cause outbreaks of pediculosis—a highly contagious condition which can result in a maculopapular rash, pruritis, and pyoderma. Also, showering at least every 7 d should have a positive effect on morale. Finally, it is impossible from available data to predict the role that sex, age, or race may play in the development and manifestation of microbial-induced skin infections for military personnel in field environments. Thus, the intervals between showers that we have recommended are assumed to be applicable to all military personnel. Additionally, the scientific and regulatory information concerning the efficacy and safety of skin-cleansing products indicate that the antimicrobial and antiseptic compounds, triclocarban and

chlorhexidine, respectively, may be the most suitable for routine use by U.S. military personnel.

Research Recommendations

The paucity of data on the relationship between hygiene and microbial skin infections makes it impossible at this time to verify or validate the assumptions and subsequent calculations we used to derive recommended maximum showering frequencies for U.S. military personnel in field environments. Therefore, we urge that research be performed to achieve such verification and validation and reduce the uncertainty in our recommendations. The specific recommendations for study of the relationship between hygiene and microbial skin infections are presented at the end of Chapter 5 and for study of cleansing agents at the end of Appendix A. Consideration of the possible efficacy of prophylactic creams for the prevention of nonsystemic microbial infections of the skin and/or for use as a temporary substitute for regular showering is beyond the scope of the research described in this report.

The Effect of Shower/Bath Frequency on the Health and Operational Effectiveness of Soldiers in a Field Setting: Recommendation of Showering Frequencies for Reducing Performance-Degrading Nonsystemic Microbial Skin Infections

1. Introduction

Microbial diseases of the skin have adversely affected performance and caused substantial losses of manpower among military personnel deployed in the field (Pillsbury and Livingood, 1947; and Allen, 1989). At the present time, the U. S. Army makes shower facilities available to soldiers in the field on a weekly basis. This bathing regimen was established to prevent infestations of the body louse and to prevent the spread of louse-borne disease (U.S. Army, 1982; and Busvine, 1985). There is no evidence, however, that this bathing frequency is adequate to prevent microbial diseases of the skin.

Sources of Information

To determine an optimum showering frequency for military personnel in the field, we relied on information from many different sources. A number of dermatologists, clinical microbiologists, and specialists in military medicine were of great assistance to us in defining crucial issues and problems related to the project. Many key references were obtained from these individuals as well.

Additionally, we evaluated a review of hard-to-obtain archived military documents, after-action reports, and official bulletins and correspondence concerning the relationship between showering, bathing and prevention of skin disease that was prepared for the U.S. Army by Col. Troychock (1991). The principal conclusions that were reached by Troychock (1991) on the basis of the limited information contained in the reviewed reports were (1) ideally, showering/bathing should take place on a daily basis; (2) where circumstances prevent daily showering/bathing, such activity should take place at least on a weekly basis; (3) loose clothing is essential; and (4) skin integrity should be maintained to prevent minor skin problems from becoming debilitating.

We also reviewed a number of Annual Research Progress Reports from the Letterman Army Institute of Research (LAIR) at the Presidio in San Francisco, California (LAIR 1972, 1973, 1976, 1977, and 1978). These documents contained lists of scientific publications from the Dermatology Research Unit at LAIR.

The late Dr. Marion Sulzberger was head of the Dermatology Research Unit at LAIR from its inception in 1964 until 1970. His collected papers are housed at the Library of the University of California, San Francisco, and we examined this collection in search of unpublished data relevant to this project. Although his papers contain a wealth of historical and scientific data, we found no relevant material that he had not previously published in the open literature.

During the 1960's and early 1970's, the U.S. Army Medical Research and Development Command (MRDC), sponsored an array of field research projects to identify the biological and physical factors that cause and/or contribute to the development of skin diseases in the tropics. Some of this research was performed at the University of Miami in Florida. According to the results of our literature search, the majority of the U.S. Army MRDC funded research on this subject was never published in peer-reviewed scientific literature, and only a limited amount of this research appears to be available in U.S. Army reports. However, we did obtain a few of the relevant U.S. Army annual reports and scientific articles that were prepared at the University of Miami (discussed below).

Researchers from the University of Miami had a long and productive working relationship with the U.S. Army MRDC. In 1962, a team of scientists and physicians from the University of Miami spent three months in Panama with U.S. troops participating in Operation Swampfox II. The focus of their research was to identify the causative organisms of skin infections among a military population in the field, to determine the prevalence of each type of infection, and to identify the role of environmental factors in the etiology of skin disease in the tropics (Taplin *et al.*, 1965; and Taplin *et al.*, 1967). The causative organisms identified by these researchers are among those listed in Table 2-1 (see Chapter 2) of this report; the role of environmental factors in the etiology of skin disease also are discussed in Chapter 2 in the section titled "Factors that Promote Microbial Infections of the Skin."

During the Vietnam war, University of Miami scientists performed a series of field epidemiology studies that were funded by the U.S. Army and were similar in focus to those completed in Panama during Operation Swampfox II. Although our search of the DTIC data base in 1990 did not result in any citations of U.S. Army reports that covered that research, much of that work was described by Allen (1989) in the text *Skin Diseases in Vietnam*—a reference work that was of great value to us and which we cite frequently in this report. Moreover, the field-research in Vietnam provided material for a number of journal articles, many of which also are discussed in this report.

Following the Vietnam war, the University of Miami continued to receive funding from the U.S. Army MRDC to study the etiology of bacterial and fungal skin diseases in the tropics. We examined the contents of the two annual progress reports (Taplin, 1975: for the period June 1, 1973, to May 31, 1974; and Taplin, 1976: for the period June 1, 1974 to December 31, 1975) and the one final report (Taplin, 1978: for the period June 1, 1971 to May 31, 1976) describing this research, which were found as a result of our literature search. These reports contain descriptions of a clinical trial of an antimicrobial soap and a clinical trial of the antiseptic chlorhexidine (reviewed in Appendix A), as well as descriptions of infections in survivors of a plane crash in the Everglades, the role of water temperature in the development of "immersion foot syndrome," and an outbreak of furunculosis (i.e., boils) in Florida. Also presented in these reports are evaluations of various medications for the treatment of ringworm, and results of surveys of skin disease among units of the Colombian Army, and of skin infections among children in Costa Rica. Although the research funded by the U.S. Army MRDC that is described in the reports and articles that we obtained yielded a great deal of valuable information, none of this information specifically addresses showering or bathing frequency and its influence (or lack of influence) on the development of microbial diseases of the skin.

Finally, we relied on the expertise of a science librarian to identify and search all computerized data bases with material of potential interest to this project. To that end, we searched Medline (1966 to present), Biosis (1969 to present), Chemical Abstracts (1967 to present), National Technical Information Service (1964 to present), the Aerospace Data Base (1962 to present), Nuclear Science Abstracts (1948 to 1976), the DOE Energy Abstracts (1974 to present), EM Base (1974 to present), and the Defense Technical Information Center (1960 to present).

Report Organization

Our report is divided into five chapters and two appendices. Chapter 1 contains an introduction as well as a discussion of the historical importance of skin disease as a source of disability among military personnel in the field. Chapter 2 describes microbial infections of the skin and factors that promote these infections. In Chapter 3, we discuss the effectiveness of cleansing in the prevention of microbial infections of the skin. That chapter, in conjunction with the material in Appendix A, contains a comprehensive review of the efficacy of water alone and in combination with unmedicated soaps, antimicrobial soaps, and chlorhexidine antiseptic in preventing skin infections. Appendix B contains a list, accompanied by available

references, of microorganisms that cause nonsystemic diseases of the skin of potential significance to U.S. military personnel. In Chapter 4, we present the equations used to calculate an optimum showering frequency. Chapter 5 contains conclusions and recommendations for future research.

Skin Disease in a Military Setting: The Historical Perspective

Diseases of the skin have had a major impact on the health of armed forces personnel involved in recent military conflicts. There are ample data that document the extent of the disability caused by skin diseases in World War II (WW II) (Pillsbury and Livingood, 1947; Sanderson and Sloper, 1953a; and Allen, 1989). A principal cause of disabling skin disease among the U.S. Armed Forces in WW II was "overtreatment dermatitis [*sic*]" (Pillsbury and Livingood, 1947), which we interpret to mean misdiagnosis and treatment of a primary condition which exacerbated the existing skin problem. Other common causes of skin disease among U.S. military personnel during WW II were bacterial pyoderma, chronic eczematous eruptions, and fungal infections (Pillsbury and Livingood, 1947). Skin diseases were of particular significance in the tropics, where high temperatures, high humidity, an abundance of biting insects, and relatively poor sanitary conditions combined to create major problems (Pillsbury and Livingood, 1947; and Allen, 1989). In the Southwest Pacific military theater during 1944 and 1945, 75% of all patients were seen for the diagnosis and treatment of skin disease. A full 20% of all hospital admissions were due to skin disease, and 15% of these patients were ultimately evacuated to the United States for additional treatment (Pillsbury and Livingood, 1947).

British troops in Southeast Asia also experienced considerable disability from skin disease. A survey made during 1947 to 1949 of British troops in Hong Kong and Malaya found that nearly 80% of the soldiers had various dermatoses of the feet, 34% had ringworm of the body, and approximately 30% had impetigo, a bacterial infection of the skin (Sanderson and Sloper, 1953a).

In the years following WW II, evidence continued to accumulate on the potential impact of skin disease on troop strength. The French military in Vietnam suffered an "... enormous drain on manpower ..." due to skin diseases caused primarily by fungal and staphylococcal infections (Allen, 1989). Sixty percent of a British infantry battalion deployed in Malaya from 1958 to 1959 developed fungal infections of the body (Harris, 1962). Among American troops in Panama during Operation Swampfox II (1962), fungal and staphylococcal infections were important causes of disease (Taplin *et al.*, 1965 and Taplin *et al.*, 1967).

As a consequence of experiences in WW II, the U.S. Armed Forces in Vietnam were better prepared to cope with skin diseases among troops than at any previous time. Nonetheless, the data from Vietnam document the enormity of the problem and the impact that skin disease had on military operations (Sulzberger and Akers, 1969; Blank *et al.*, 1969; McMillan and Hurwitz, 1969; Allen *et al.*, 1971; Allen *et al.*, 1972; Allen and Taplin, 1973; and Allen, 1989).

During the period 1965 to 1972, skin diseases were the third leading cause of hospital admissions for non-battle injuries in Vietnam (Allen, 1989). These figures do not accurately represent the true scope of the problem, however. Allen (1989) estimated that less than 1% of those who sought treatment for skin disease were hospitalized. During the same eight-year period (1965 to 1972), 1,412,500 outpatient visits for treatment of dermatological disorders were documented, making skin disease " ... the single greatest cause of outpatient visits ... " during the Vietnam war (Allen, 1989). Of even greater significance, however, is the fact that these diseases were frequently disabling and dramatically affected combat-troop strength. Allen (1989) cites as an example the records of the U.S. Army 9th Infantry Division between 1968 and 1969; 47% of the man-days lost (including those due to battle wounds), or a total of 26,861 days, were due to skin infections.

Cutaneous fungal infections, due primarily to *Trichophyton mentagrophytes*, were the most common cause of skin disease among troops in Vietnam (Blank *et al.*, 1969; Allen *et al.*, 1972; Allen and Taplin, 1973; and Allen, 1989). Other pathogenic fungi isolated by Allen *et al.* (1972) and identified as important causes of dermatophytosis in Vietnam include *Epidermophyton floccosum*, *Trichophyton rubrum*, and *Candida albicans*. Staphylococcal and streptococcal infections of the skin were also a major cause of morbidity among American troops in Vietnam (Blank *et al.*, 1969; McMillan and Hurwitz, 1969; and Allen *et al.*, 1971).

According to the data from Vietnam cited above, for *Trichophyton mentagrophytes*, the groin, ankle, and foot were the most common sites of infection; candidiasis was largely restricted to the groin and toe web. Additionally, the majority of bacterial infections occurred on the ankle and dorsum of the foot where insect or leech bites and maceration of the skin from wet socks and boots compromised the integrity of the skin.

The Role of Environment in the Development of Microbial Skin Disease in Military Personnel

The environment undoubtedly was a major factor in the prevalence and severity of skin disease in combat troops—both in WW II and Vietnam. In the hot, humid climate of the tropics, sweat and other moisture (e.g., from rainfall) does not evaporate readily from the body. When skin remains moist for extended periods of time under clothing and boots, a microenvironment of high heat and humidity is created that is conducive to the proliferation of microorganisms (R. Marples, 1965; Bibel and LeBrun, 1975; and Aly *et al.*, 1978). Occlusion of the skin from clothing and footwear was of singular importance in the development of microbial diseases of the skin in Vietnam (McMillan and Hurwitz, 1969; Allen *et al.*, 1972; and Allen, 1989). For example, fungal infections were largely restricted to those sites on the body where occlusion was most pronounced—the groin, toe web, ankle, and dorsum of the foot (Blank *et al.*, 1969; McMillan and Hurwitz, 1969; Allen *et al.*, 1972; and Allen and Taplin, 1973). Among British troops in Malaya (Sanderson and Sloper, 1953a) and American troops in Vietnam (Allen, 1989), there was a close concordance between the incidence of skin disease, and rainfall and humidity (i.e., as rainfall and relative humidity increased, there was a corresponding increase in the incidence of skin disease). Sulzberger and Akers (1969) cited data from an infantry division deployed in the Mekong Delta of Vietnam that showed the number of combat man-days lost due to skin disease varied in direct relation to the season. In the dry season, 18% of all combat man-days lost were due to skin disease; this increased to approximately 70% during the rainy season.

A preliminary report from the recent war in the Persian Gulf (Gasser *et al.*, 1991) is notable in that there is little mention of skin disease among the troops. However, military personnel were initially deployed during the hot summer months prior to the actual military engagement, which occurred during the cooler months of fall and winter. Accordingly, the apparent absence of any skin disease might be explained by one or all of the following factors: (1) improved health care (relative to Vietnam), (2) activity schedules during cooler periods of the day in summer, (3) a short period of conflict in the relatively cool and dry fall and winter, and/or (4) an incomplete review of all relevant medical data for the military personnel deployed in the region during this time.

2. Microbial Infections of the Skin

Under normal conditions, healthy, intact human skin rarely becomes infected. For infection to develop, pathogenic microorganisms require a route of entry into subsurface tissues such as might be provided by a minor wound, abrasion, insect bite, or the action of microbial toxins (Marples, 1976). Many common pathogens (e.g., *Staphylococcus aureus*, *Streptococcus pyogenes*) produce cytolytic toxins and enzymes capable of damaging intact human skin (Stanier *et al.*, 1976); however, these pathogens must reach a threshold-population level to produce locally damaging quantities of toxins that may breach the integrity of the skin (Marples, 1976). Many factors, such as competition from the resident flora and the antimicrobial properties of skin-surface lipids, generally prevent pathogenic microorganisms from reaching damaging levels (Marples, 1976; and Aly *et al.*, 1975). Potentially pathogenic microorganisms such as *Staphylococcus aureus* and *Candida albicans* can exist transiently on the skin, however, and certain people become carriers of these pathogens with no overt evidence of disease. Nevertheless, these microorganisms, as well as other skin pathogens, do cause significant morbidity under certain conditions. Defining these conditions and describing how they contribute to the development of skin disease is the purpose of this chapter.

Resident and Pathogenic Microorganisms

The human skin supports an abundant microflora. Qualitatively, the resident flora (i.e., those microorganisms able to multiply on the skin rather than simply survive there) is relatively uniform (Price, 1938). Resident bacteria include staphylococci, micrococci, corynebacteria, brevibacteria, and propionibacteria; several yeasts and fungi are also commonly found (M. Marples, 1965; Noble, 1981; and Aly, 1985). The ecological requirements, distribution, and population densities of many of these microorganisms have been described by M. Marples (1965). Microbial growth is favored by moisture; the highest numbers of resident bacteria are found on relatively moist intertriginous skin (i.e., apposed surfaces of the skin such as creases of the neck, and folds of the groin and armpit) while the lowest numbers are found on the dry regions of the human body such as the hands and legs (M. Marples, 1965; Marples, 1976; Aly and Maibach, 1977; and Aly *et al.*, 1979).

Both pathogenic and nonpathogenic microorganisms make up the transient flora of human skin. These microorganisms are continuously deposited on skin as a result of transfer from the nasal passages, the gut, and the external environment. Many of these microorganisms can survive temporarily on the surface of the skin,

generally becoming established only if the skin is altered by injury or disease (Marples and Leyden, 1985). However, some potentially pathogenic microorganisms such as *Staphylococcus aureus* and *Candida albicans* regularly become established on human skin. For example, it has been determined that between 10 and 45% of the population carries *Staphylococcus aureus* in the nose and/or perineal (skin covering pelvic floor) area (Williams, 1963; and Noble, 1981). Aly *et al.* (1979) found a vulvar carriage rate of *Staphylococcus aureus* of 67%. Similarly, the yeast *Candida albicans*, a resident of the alimentary tract, is carried in the vagina of approximately 5% of healthy, nonpregnant women, and on the skin of 3 to 10% of young adults (Noble, 1981). The existence of these potential pathogens in the absence of disease indicates that without promoting factors such as occlusion or wounding, baseline populations of these organisms are not sufficient to cause infection.

Microbial infections of the skin caused significant decreases of combat troop strength in WW II and Vietnam (see Chapter 1). Data from these two conflicts consistently implicate a relatively small number of microorganisms as the cause of the vast majority of skin disease (i.e., the fungi *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Trichophyton rubrum*, and *Candida albicans*, and the bacteria *Staphylococcus aureus* and *Streptococcus pyogenes*). These and other microorganisms that cause nonsystemic diseases of the skin are listed in Table 2-1.

Factors that Promote Microbial Infections of the Skin

Intact human skin is a formidable protective barrier between the external environment and the internal tissues of the human body. Microbial infections of the skin develop when a combination of factors exist; the presence of a particular pathogenic microorganism is only one of these. The integrity of the skin must also be altered—by abrasion or wounding, or by occlusion and hydration (which can promote the growth of pathogenic microorganisms and lead to an accumulation of cytolytic toxins). Furthermore, contact with soil, sweat, feces, and/or urine may facilitate microbial infections of the skin. Racial differences in susceptibility to infection also have been considered as possible etiologic factors in the development of microbial infections of the skin.

Wounding

Early attempts to develop an experimental model of bacterial skin infections centered on various means of introducing bacteria into subsurface tissues. These

Table 2-1. Microorganisms that may induce skin diseases and therefore may be of significance to the U.S. military (see Appendix B for more detail).

Type of microorganism	Scientific name
Bacteria	<p> <i>Acinetobacter calcoaceticus</i> <i>Actinomyces israelii</i> <i>Aeromonas hydrophila</i> <i>Aeromonas aerogenes</i> <i>Bacterioides corrodens</i> <i>Bacterioides fragilis</i> <i>Bacterioides melaninogenicus</i> <i>Bartonella bacilliformis</i> <i>Clostridium perfringens</i> <i>Clostridium welchii</i> <i>Corynebacterium acnes</i> <i>Corynebacterium diphtheriae</i> <i>Corynebacterium minutissimum</i> <i>Corynebacterium tenuis</i> <i>Enterobacter cloacea</i> <i>Enterobacter aerogenes</i> <i>Erysipelothrix rhusiopathiae</i> <i>Escherichia coli</i> <i>Francisella tularensis</i> <i>Hemophilus spp.</i> <i>Klebsiella pneumoniae</i> <i>Mycobacterium ulcerans</i> <i>Mycobacterium marinum</i> <i>Pasteurella multocida</i> <i>Peptococcus spp.</i> <i>Peptostreptococcus spp.</i> <i>Proteus mirabilis</i> <i>Pseudomonas aeruginosa</i> <i>Pseudomonas cepacia</i> <i>Pseudomonas maltophilia</i> <i>Pseudomonas pseudomallei</i> <i>Serratia marcescens</i> <i>Staphylococcus aureus</i> <i>Staphylococcus cohnii</i> </p>

Table 2-1. continued.

Type of microorganism	Scientific name
Bacteria (continued)	<i>Staphylococcus epidermidis</i>
	<i>Staphylococcus saprophyticus</i>
	<i>Staphylococcus warneri</i>
	<i>Streptobacillus moniliformis</i>
	<i>Streptococcus bovis</i>
	<i>Streptococcus faecalis</i>
	<i>Streptococcus pneumoniae</i>
	<i>Streptococcus pyogenes</i>
	<i>Streptococcus salivarius</i>
Fungi	<i>Candida albicans</i>
	<i>Candida tropicalis</i>
	<i>Phaeoannellomyces wernecki</i> (formerly identified as <i>Cladosporium wernecki</i>)
	<i>Cladosporium mansonii</i>
	<i>Epidermophyton floccosum</i>
	<i>Microsporum audouinii</i>
	<i>Microsporum canis</i>
	<i>Microsporum distortum</i>
	<i>Microsporum ferrugineum</i>
	<i>Microsporum gypseum</i>
	<i>Pityrosporon orbiculare</i>
	<i>Pityrosporon ovale</i>
	<i>Trichophyton concentricum</i>
	<i>Trichophyton ferrugineum</i>
	<i>Trichophyton mentagrophytes</i>
	<i>Trichophyton rubrum</i>
	<i>Trichophyton schoenleini</i>
<i>Trichophyton tonsurans</i>	
<i>Trichophyton verrucosum</i>	
<i>Trichophyton violaceum</i>	
<i>Trichosporon capitatum</i> (see Appendix B for reference)	
Viruses	<i>Herpes simplex</i>
	<i>Papilloma spp.</i>
Other	<i>Leishmania tropica</i>

measures included the use of intradermal injections and the insertion of contaminated silk sutures into subcutaneous tissues (Elek, 1965); the use of puncture wounds, scratches, superficial abrasions, induction of allergic contact dermatitis followed by stabbing with a lancet, and the insertion of bacteria-contaminated polyethylene granules into minor wounds (Duncan *et al.*, 1970); and skin stripping with cellophane tape (Marples and Kligman, 1972; and Duncan *et al.*, 1970). In general, these experiments had relatively poor success in consistently producing infections. Nonetheless, empirical data from Costa Rica (Taplin *et al.*, 1973), Panama (Taplin *et al.*, 1965 and 1967; and Allen and Taplin, 1974), Trinidad (Sharrett *et al.*, 1974) and Vietnam (McMillan and Hurwitz, 1969; Allen *et al.*, 1971; Allen and Taplin, 1973; and Allen, 1989) have consistently implicated skin trauma as an essential precursor to the development of bacterial infections of the skin. The role of minor wounds or abrasion in the pathogenesis of fungal infections of the skin is not well understood.

Occlusion

Although wounds provide access to subsurface tissues and a moist nutrient-rich environment conducive to bacterial growth, skin trauma alone is not necessarily sufficient for pathogenic bacteria to attain populations capable of causing disease. Occlusion has been repeatedly identified as being of equal or greater significance than the presence of a minor wound in the development of bacterial infections of the skin, and appears to be crucial to the development of fungal infections of the skin as well (McMillan and Hurwitz, 1969; Allen *et al.*, 1972; Allen and Taplin, 1973; Maibach and Kligman, 1962; Rebora *et al.*, 1973; Marples and Kligman, 1972; and Reinhardt *et al.*, 1974).

Occlusion of the skin with a relatively impermeable plastic film alters the microbial ecology of the skin surface. One of the immediate effects of occlusion is an increase in skin-surface moisture (R. Marples, 1965; Hojyo-Tomoka *et al.*, 1973; and Aly *et al.*, 1978). For the first several days of occlusion, bacterial counts increase many-fold, but these begin to decline after four days (Bibel and LeBrun, 1975; and Aly *et al.*, 1978). Faergemann *et al.* (1983) showed that populations of the yeast *Pityrosporon orbiculare* also increase during occlusion over a 3-d period.

Under occlusion there is an increase in skin pH, transepidermal water loss (i.e., evaporative water loss from the skin surface), water content of the stratum corneum, and skin-surface CO₂ emission rate (Aly *et al.*, 1978; and Aly, 1982). Although the specific role of each of these variables in the enhanced rate of microbial growth has not been elucidated, the increase in transepidermal water loss and the increase in

hydration of the stratum corneum are thought to promote the growth and pathogenicity of microorganisms (Maibach and Kligman, 1962; and Marples and Kligman, 1972).

In the absence of experimentally applied occlusive barriers, hydration of the stratum corneum is affected by sweat production and by the evaporation of sweat and other moisture from the skin surface (Taplin *et al.*, 1965; and R. Marples, 1965). Among the variables that influence evaporation of skin-surface moisture are ambient temperature, relative humidity, and clothing. Hatch *et al.* (1990) have shown that certain clothing fabrics can create an occlusive barrier that alters several physiological parameters, especially during exercise in a hot and humid environment. For example, female volunteers who wore either polyester or 100% cotton T-shirts for 20 min in a hot and humid environment (85°F, 75% relative humidity) exhibited a slight increase in stratum corneum water content, transepidermal water loss, and capillary blood flow. [Similar results also were obtained in an earlier experiment by Hatch *et al.* (1987) that involved occlusion of the forearm with triacetate and polyester fabric.] However, forty minutes of vigorous exercise under the same environmental conditions resulted in a two-fold increase in stratum corneum water content, a 23-fold increase in evaporative water loss from the skin surface, and a slightly less than two-fold increase in capillary blood flow, compared to baseline values. These differences were statistically significant. These data indicate that clothing has occlusive properties, and, when worn during exercise in a hot, humid environment, can produce physiological changes and a skin microclimate similar to that induced experimentally by occlusion.

There is no evidence, however, that occlusion of the skin by clothing creates a health problem for military personnel other than when troops are stationed in the tropics and are on patrol for prolonged periods of time (Taplin *et al.*, 1965; Allen and Tapiin, 1973; and Allen, 1989). In the tropics, moisture does not evaporate readily from bare skin, and evaporation is further restricted by clothing. When skin is kept moist for extended periods of time the stratum corneum becomes hydrated and loses its integrity, making the skin more susceptible to microbial infections (R. Marples, 1965). In nontropical environments, there is less humidity to dampen clothing and boots, and skin-surface moisture is able to evaporate even though clothing restricts the process to some extent.

Another equally significant variable in the development of microbial skin infections is the period of time that skin remains occluded. Under noncombat conditions, military personnel are free to shower frequently, change clothes

frequently, and sleep in unrestrictive clothing (or none at all). These behavior patterns keep the accumulation of microorganisms, sweat, and dirt on the skin to a minimum. Such personal habits also serve to minimize the period of time occlusive clothing is worn, which can be a significant factor in producing adverse pathological and ecological changes of the skin. Hojyo-Tomoka *et al.* (1973) clearly showed that intermittent occlusion of the skin without added moisture does not produce the physiological or microbial changes that continuous occlusion or intermittent occlusion with superhydration does. Complete occlusion of the forearm seven hours a day for two weeks did not cause a significant change in the total number of aerobic bacteria and no overt changes in the skin surface were observed. However, the same procedure carried out with the addition of a wet felt pad caused dramatic increases in populations of aerobic bacteria (two to four orders of magnitude). Implicit in these data is the fact that under occlusion, time and moisture are both significant factors in microbial growth and pathological changes, with one of the crucial endpoints being hydration of the stratum corneum of the skin.

Contact with Soil, Sweat, Feces, and/or Urine

Soil, sweat, feces, and/or urine may contribute to microbial infections of the skin in military personnel in the field. The limited data implicating these factors are described below.

Soil. Allen (1989) identified dirt (which we presume to be soil) as one of many factors that contributed to bacterial infections of the skin among military personnel in Vietnam. With respect to furuncles (boils) caused by *Staphylococcus aureus*, Allen (1989) noted that "Sweat, grime, and contact with lubricants were thought to be important factors ...". Soil is a reservoir of some microorganisms that are potential pathogens of human skin (e.g., *Pseudomonas cepaciae*). There is no evidence, however, that soil-borne microorganisms can initiate infections on healthy, intact human skin. However, physical activity in the presence of accumulated dirt and grime could lead to abrasion of the skin and provide a route of entry for disease-producing microorganisms.

Sweat. Although there are no data that specifically define the relationship between sweat, the growth of pathogenic microorganisms, and induction of microbial infections of the skin, sweat may contribute to the development of microbial infections of the skin (see Allen, 1989). For example, the growth of bacteria and fungi on the surface of the skin generally is favored by relatively high moisture; resident microorganisms occur in the highest numbers on intertriginous regions of the body

kept moist (in part) by sweat (Aly and Maibach, 1977; and Noble, 1981). Observations made in the field and reported by Allen (1989) implicated sweat as a factor contributing to microbial diseases of the skin among military personnel deployed in tropical environments. Sanderson and Sloper (1953b) also are cited by Allen (1989) as having speculated that maceration of the skin (degenerative changes in skin integrity as a consequence of prolonged contact with moisture) from unevaporated sweat was a factor in the development of fungal infections of the skin among British troops in Malaya. Supporting these observations and speculation are data from two experimental studies. First, R. Marples (1965) reported that prolonged occlusion of the skin (i.e., 4 d) by an impermeable-plastic barrier leads to hydration of the stratum corneum and ultimately to deterioration of skin integrity. Second, Hatch *et al.* (1990) showed that unevaporated sweat that remains in contact with the skin, even under typical clothing material (e.g., cotton or polyester fabric), leads to some degree of hydration of the stratum corneum. Consequently, if physical activity takes place in a hot, humid environment where sweat cannot evaporate, the skin is likely to be vulnerable to maceration and/or abrasion from sweat-dampened clothing, which may provide a route of entry for microbial pathogens.

Feces. We found no published data on the relationship between the presence of human fecal material on skin and subsequent development of irritation and/or microbial infections of the skin. Human feces, however, contain microorganisms that are potential pathogens of human skin (e.g., *Candida albicans*, and *Escherichia coli*). Accordingly, fecal material represents a reservoir of potential skin pathogens, which could contribute to skin infections—both in the perineal area and on other parts of the body. In the absence of any data describing the relationship between skin contact with fecal material and subsequent microbial infection, we can only assume that adherence to a personal hygiene regimen (e.g., daily cleansing) will minimize the period of time feces may be in contact with the skin and thereby limit the risk of skin infection from fecal contamination.

Urine. The health consequences of prolonged skin contact with urine are not well defined. Infants spend the first year or two of life in diapers. Frequent urination, in combination with the occlusive barrier created by the diaper, produces a moist microenvironment that makes the skin of a diapered area susceptible to “insult” (Boisits and McCormack, 1982). Consequently, diaper rash is a common ailment of infants (Leyden *et al.*, 1977; Leyden and Kligman, 1978; Boisits and McCormack, 1982; and Jordan and Blaney, 1982). Although diaper rash is currently recognized as a multifactorial disease, the most common form is that caused by occlusion and chafing

(Leyden *et al.*, 1977; Leyden and Kligman, 1978; Boisits and McCormack, 1982; and Jordan and Blaney, 1982).

A widely accepted early theory on the cause of diaper rash suggested that microorganisms capable of releasing ammonia from urea were responsible for the disease (Cooke, 1921). Although certain forms of diaper rash are, in fact, caused or exacerbated by microorganisms (e.g., diaper rash due to atopic dermatitis), Leyden and Kligman (1978) found no statistical differences in the skin microflora between healthy infants and those with a "chafing form" of diaper rash. This form of diaper rash appears to develop as a consequence of maceration of continually moist skin and reportedly responds to "drying measures" (which we interpret to be the absence of a diaper, addition of a desiccant, etc.) and frequent changes (Leyden and Kligman, 1978). Although urine is the principal source of the moisture contributing to development of the chafing form of diaper rash, the most important etiological factor appears to be moisture that remains in contact with the skin for prolonged periods of time and not any constituent of urine itself.

Separately, Leyden *et al.* (1977), showed that there were no significant differences in ammonia levels in the diapers from healthy infants and from infants with a chafing form of diaper rash. Additionally, no difference was detected among the two groups of infants with respect to the prevalence of bacteria capable of releasing ammonia from urea.

The irritant properties of urine were examined by Leyden *et al.* (1977) in a series of three experiments. In the first experiment, parental consent was obtained to apply samples of ammonia-enriched urine [i.e., an ammonia concentration of approximately 16 parts per thousand (ppt)] to sterile gauze, which was then sealed against the buttocks of 10 healthy infants and left in place for 24 h. The second experiment was designed to determine if repeated application of urinary ammonia could irritate the skin. Leyden *et al.* (1977) applied either ammonia-enriched urine or solutions of 2.5, 5, or 10% ammonia hydroxide to occlusive patches and then placed these patches on the forearm of adult subjects. These patches were "removed daily" each day for 5 d so that the skin beneath the occlusive patch could be examined. Leyden *et al.* (1977) did not specify whether or not the patches were resaturated with the test solution of ammonia at the time of reapplication. Additionally, the skin of 10 adult volunteers was scarified (i.e., abraded), and solutions of 0.5 or 5 ppt of urinary ammonia were placed on the scarified skin each day for a period of 3 d; the skin was occluded after each application. Finally, Leyden *et al.* (1977) attempted to determine whether urinary ammonia may damage skin in a manner that would make it more

susceptible to damage resulting from frictional contact with a diaper fabric. In this third experiment involving adult volunteers, normal skin, skin exposed for 24 h to a water-soaked gauze pad, and skin exposed for 24 h to a gauze pad saturated with urinary ammonia (either at a concentration of 0.5 or 20 ppt) was abraded with a motor-driven rod with a hemispheric tip covered with diaper material.

In the first experiment, none of the infants were reported to have exhibited erythema or any other visible changes in the skin at the site of application of the occlusive urine-soaked patch. In the second experiment, the adult volunteers generally were without adverse effects. However, the treated site of one of the adults exposed to a solution of 10% ammonia hydroxide did display some erythema after 48 h. Scarification of the skin in combination with daily application of urinary ammonia produced pronounced erythema at treated sites of the adult volunteers at the end of 3 d. These results indicate that urine may well act as an irritant to damaged skin. Leyden *et al.* (1977) also described a marked difference in the number of strokes required to abrade normal skin compared to that required to abrade skin hydrated with water or with either of the two different concentrations of urinary ammonia. However, there was no significant difference reported in susceptibility to abrasion for either the skin hydrated with water or for the skin hydrated with ammonia.

Therefore, the studies by Leyden *et al.* (1977) indicate that urine does not appear to be an irritant to intact adult skin, even when contact between the skin and the urine was maintained for up to 5 d. However, these studies also demonstrate that skin may be more susceptible to "frictional" damage if it remains in contact with moisture, regardless of the source of that moisture (e.g., urine or water), and that damaged (i.e., scarified) skin in contact with urine for up to 3 d may be susceptible to irritation.

Racial Differences

Racial differences in skin structure and the susceptibility of the skin to microbial infections and chemical irritation have been investigated [e.g., cellular structure of the stratum corneum by Thomson (1955) and Weigand *et al.* (1974); infections due to streptococci by Allen *et al.* (1971); infections due to *Staphylococcus aureus* by Duncan *et al.* (1981); infections due to *Candida albicans* by Rebora *et al.* (1973), and Rebora and Guarrera (1988); and response to chemical irritants by Weigand and Gaylor (1974), and Berardesca and Maibach (1988 and 1991)]. However, based on the limited nature of these data and on the absence of any strong statistical evidence to indicate otherwise, racially based differences in the structure or susceptibility of the skin to microbial

infections or chemical irritants do not have any practical significance at this time for the development of an optimum showering frequency for military personnel.

Development of Skin Infections

The complex processes involved in the development of skin infection have been examined in a series of experiments in which investigators attempted to define the many variables that contribute to the development of disease. These experiments have generally used protocols in which inocula of pathogenic microorganisms were applied to the skin and observed for a specific period of time.

Table 2-2 presents a summary of data on the organisms and experimental conditions that have been used successfully to induce infections of the skin. In all cases, occlusion of the inoculated site(s) to prevent desiccation and die-off (Norton and Novy, 1931; Rebell *et al.*, 1950; Maibach and Kligman, 1962; Marples, 1976; and Leyden *et al.*, 1980b) was found to be essential for the survival and growth of microorganisms.

Experimental Infection with *Candida albicans*

Maibach and Kligman (1962) induced infection with *Candida albicans* by applying a suspension of organisms to the skin of the anterior thigh of male volunteers, and covering the inoculated site with tape or bandage material. Evidence of infection was apparent in 36 to 72 hours (Table 2-2). Maibach and Kligman (1962) found that infection did not develop unless the site was occluded, even when "massive numbers" of *Candida albicans* were applied daily to the same area for seven days. These experiments defined a dose-response relationship between the number of organisms in the inoculum and initiation of infection. Application of 1.5×10^7 cells/cm² induced infection in 50% of the subjects; 3.8×10^7 cells/cm² yielded infection in all participants. Rebera *et al.* (1973) also succeeded in inducing infections with *Candida albicans*, but with substantially fewer organisms and in a shorter period of time (24 h). Using a protocol similar to that of Maibach and Kligman (1962), Rebera *et al.* (1973) confirmed that the efficacy with which infection could be initiated depended on the size of the inoculum; as the number of *Candida albicans* in the inoculum increased from 2.5×10^2 cells/cm², to 2.5×10^3 cells/cm², to 2.5×10^4 cells/cm², infections were observed in 65%, 90%, and 95% of the subjects, respectively. Rebera *et al.* (1973) recovered a relatively small number of *Candida albicans* at the onset of infection during these experiments (i.e., 1.6×10^3 cells/cm² to 3.2×10^4 cells/cm²; see Table 2-2), indicating that the applied inoculum approached the population threshold for producing infection.

Table 2-2. Experimental induction of nonsystemic skin infections in human volunteers.

Microorganism ^a	Population/cm ²		Time to infection or end of study (h)	Site of inoculation	Volunteer population	Experimental conditions	Results	Reference
	Inoculum	Infection						
<i>C. albicans</i>	1.5×10^7	N/A ^b	36 to 72	thigh	male prisoners	occlusion	infection in 50% of subjects	Maibach and Kligman (1962)
<i>C. albicans</i>	3.8×10^7	N/A ^b	36 to 72	thigh	male prisoners	occlusion	infection in 100% of subjects	Maibach and Kligman (1962)
<i>C. albicans</i>	2.5×10^2	1.6×10^3	24	forearm	male prisoners	occlusion	infection in 65% of subjects	Rebora <i>et al.</i> (1973)
<i>C. albicans</i>	2.5×10^3	2.4×10^4	24	forearm	male prisoners	occlusion	infection in 90% of subjects	Rebora <i>et al.</i> (1973)
<i>C. albicans</i>	2.5×10^4	3.2×10^4	24	forearm	male prisoners	occlusion	infection in 95% of subjects	Rebora <i>et al.</i> (1973).
<i>P. aeruginosa</i>	8.3×10^4	1.6×10^6	48	forearm	males	occlusion	no infection	Leyden <i>et al.</i> (1980a)
<i>P. aeruginosa</i>	8.3×10^4	1.5×10^6	168	forearm	males	occlusion	infection in 5% of subjects	Leyden <i>et al.</i> (1980a)

Table 2-2. continued.

Microorganism ^a	Population/cm ²		Time to infection or end of study (h)	Site of inoculation	Volunteer population	Experimental conditions	Results	Reference
	Inoculum	Infection						
<i>P. aeruginosa</i>	8.3×10^4	8.5×10^6	48	forearm	males	wet dressing; occlusion	infection in 23% of subjects	Leyden <i>et al.</i> (1980a)
<i>P. aeruginosa</i>	8.3×10^4	9.5×10^6	168	forearm	males	wet dressing; occlusion	infection in 68% of subjects	Leyden <i>et al.</i> (1980a)
<i>S. aureus</i>	4.0×10^2	2.0×10^7	144	forearm	male prisoners	occlusion after degerming with alcohol	infection in 33% of subjects	Singh <i>et al.</i> (1971)
<i>S. aureus</i>	1.5×10^4	2.0×10^7	96	forearm	male prisoners	occlusion after degerming with alcohol	infection in 60% of subjects	Singh <i>et al.</i> (1971)
<i>S. aureus</i>	3.7×10^4	2.9×10^6	96	forearm	male prisoners	occlusion after degerming with alcohol	infection in 80% of subjects	Singh <i>et al.</i> (1971)
<i>S. aureus</i>	2.0×10^7	2.0×10^7	N/A ^b	forearm	male prisoners	occlusion after degerming with alcohol	infection in 94% of subjects	Singh <i>et al.</i> (1971)

Table 2-2. continued.

Microorganism ^a	Population/cm ²		Time to infection or end of study (h)	Site of inoculation	Volunteer population	Experimental conditions	Results	Reference
	Inoculum	Infection						
<i>S. aureus</i>	4.0×10^3	1.6×10^6	24	forearm	male prisoners	occlusion applied 24h after wounding	infection in 60% of subjects	Marples and Kligman (1972)
<i>S. pyogenes</i>	5.0×10^5	7.7×10^4	24	forearm	males	bacteria rubbed into wounded skin; occlusion	infection in 100% of subjects	Leyden <i>et al.</i> (1980b)
<i>T. mentagrophytes</i>	1.0×10^2	N/A ^b	96	forearm	males	occlusion	infection in 100% of subjects	Reinhardt <i>et al.</i> (1974)
<i>T. mentagrophytes</i>	1.0×10^4	N/A ^b	96	ankle	males	occlusion	infection in 100% of subjects	Reinhardt <i>et al.</i> (1974)
<i>T. mentagrophytes</i>	3.0×10^2	N/A ^b	96	forearm	males and females	wet dressing; occlusion	infection in 90% of subjects	Aly <i>et al.</i> (1991)

^a *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Trichophyton mentagrophytes*.

^b N/A indicates the information is not available.

Experimental Infection with *Staphylococcus aureus*

Staphylococcus aureus is one of the most common bacterial pathogens of human skin, and there has been considerable interest in experimentally inducing infection with this organism (e.g., Foster and Hutt, 1960; Elek, 1965; Duncan *et al.*, 1970; Singh *et al.*, 1971; and Marples and Kligman, 1972). Table 2-2 includes data only from Singh *et al.* (1971) and Marples and Kligman (1972) as these two papers were the most comprehensive.

Singh *et al.* (1971) were able to induce superficial infections with *Staphylococcus aureus* by first degerming the skin with alcohol, applying an inoculum of the bacteria, and occluding the area for 4 d. The number of infections induced in volunteers was clearly related to the inoculum size (see Table 2-2). Populations of *Staphylococcus aureus* associated with infection varied from 2.9×10^6 cells/cm² to 2×10^7 cells/cm². The importance to the development of infection of degerming the skin with alcohol is not entirely clear. Although alcohol killed some resident bacteria (potential competitors), substantial numbers remained on the skin. An alternative explanation is that the alcohol removed skin lipids whose antimicrobial properties may have acted to inhibit the growth of *Staphylococcus aureus* (Aly *et al.*, 1975).

Marples and Kligman (1972) developed a protocol for producing infection with *Staphylococcus aureus* on superficially wounded skin—which they considered to be a more realistic model for infection than that used by Singh *et al.* (1971). Their basic technique was to strip away the stratum corneum with cellophane tape, apply *Staphylococcus aureus* 24 to 48 h later, then occlude the site for 24 h. Use of this approach with an inoculum of 4×10^3 cells/cm² resulted in infection in 60% of volunteers (Table 2-2). In the process of developing a safe experimental procedure, Marples and Kligman (1972) immediately occluded freshly wounded skin that was inoculated with 10^5 *Staphylococcus aureus*. Infection developed rapidly (within 6 h) in all five subjects, demonstrating the pathogenicity of *Staphylococcus aureus* under optimal conditions.

Experimental Infection with *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is ubiquitous in the environment, but does not commonly infect the skin of healthy humans. For burn victims and those with a compromised immune system however, *Pseudomonas aeruginosa* is highly pathogenic (Hall *et al.*, 1968). Hojyo-Tomoka *et al.* (1973) have shown a correlation between the presence of large numbers of *Pseudomonas aeruginosa* (10^6 to 10^7 cells)

and development of a papulovesicular rash when wet dressings were maintained (with occlusion) on the skin of volunteers for 10 d. Occlusion alone did not generally result in infection. Presumably, the relatively high populations of *Pseudomonas aeruginosa* that were observed in these experiments were of environmental origin, because bacteria were not applied experimentally to skin as an inoculum. Singh (1973) applied inocula of *Pseudomonas aeruginosa* (8.9×10^1 cells/cm² to 2×10^5 cells/cm²) to alcohol-degermed skin, followed by 6 d of occlusion. He was unsuccessful, however, in inducing infection.

To define the experimental conditions conducive to development of infection from *Pseudomonas aeruginosa*, Leyden *et al.* (1980a) applied inocula under occlusion, or occlusion with wet dressings (see Table 2-2). Their data confirm the preference of *Pseudomonas aeruginosa* for wet environments and the relatively long period of time required for infection to develop. The most notable success was achieved when inocula of *Pseudomonas aeruginosa* were maintained under wet dressings for 7 d (Table 2-2). Occlusion of *Pseudomonas aeruginosa* for 2 d in the absence of wet dressings did not provoke a response; by 7 d, infection developed in a single volunteer. Inoculation of *Pseudomonas aeruginosa* (1×10^3 cells/cm² to 1×10^9 cells/cm²) onto scarified skin (which was then covered with an adhesive bandage) produced an inflammatory response in the majority of subjects within 24 h.

Experimental Infection with *Streptococcus pyogenes*

Streptococcus pyogenes was one of the most pervasive bacterial pathogens producing infection among American troops in Vietnam. Glomerulonephritis can develop as a sequel to streptococcal skin infections, and in certain locales (e.g., Trinidad) epidemics have occurred (Poon-King *et al.*, 1967). Sharrett *et al.* (1974) and Taplin *et al.* (1973) identified poor hygiene and environmental factors (heat, humidity, biting insects) as contributing to the prevalence of streptococcal infections in Trinidad and Colombia, respectively, and both groups observed a correlation between minor skin trauma and the eventual development of infections with *Streptococcus pyogenes*. Sharrett *et al.* (1974) and Taplin (1981) readily recovered streptococci from children's skin, and Dudding *et al.* (1970) speculated that colonization of normal skin with *Streptococcus pyogenes* occurs prior to the development of overt infection.

Leyden *et al.* (1980b) found that it was extremely difficult to experimentally induce superficial skin infections with *Streptococcus pyogenes*. Inoculation of bacteria onto normal skin, on lipid-rich and lipid-poor areas of the body, and on skin degermed with alcohol did not result in infection even when occlusion was

maintained for 48 h. In all instances, populations of *Streptococcus pyogenes* declined rapidly after inoculation, and by 48 h none could be recovered from the skin of study participants. However, superficial scarification of the skin and inoculation of 5×10^5 *Streptococcus pyogenes* per cm^2 , followed by rubbing the bacteria into the wound and occluding the area for 24 h resulted in infection in all participants (Table 2-2). Populations of *Streptococcus pyogenes* assessed at 24 h post-inoculation showed a substantial decline relative to the inoculation population. Leyden *et al.* (1980b) concluded that *Streptococcus pyogenes* is unable to survive or colonize normal, intact skin, and the reported prevalence of these bacteria on the skin (e.g., Sharrett *et al.*, 1974; Taplin *et al.*, 1973; and Taplin, 1981) reflected transmission from open lesions and environmental reservoirs.

Experimental Infection with *Trichophyton mentagrophytes*

Skin infections caused by *Trichophyton mentagrophytes* reached epidemic proportions among American troops in Vietnam (Blank *et al.*, 1969; and Allen and Taplin, 1973). *Trichophyton mentagrophytes* also caused significant morbidity in British troops in southeast Asia (Sanderson and Sloper, 1953a; and Harris, 1962). Allen and Taplin (1973) identified high temperature and humidity, prolonged exposure to water or wet clothing, and maceration and occlusion of the skin as factors that predisposed troops to infection with *Trichophyton mentagrophytes*. The experimental model of *Trichophyton mentagrophytes* infection developed by Reinhardt *et al.* (1974) reproduced many of the conditions identified by Allen and Taplin (1973) as important factors in *Trichophyton mentagrophytes*-induced infections. In a pilot experiment, patches of fabric were cut from military-issue socks, sewn into the uppers of "jungle boots," and saturated with a suspension of *Trichophyton mentagrophytes* equivalent to 4×10^4 spores per cm^2 . These boots were worn continuously by volunteers for five days. Sterile water was added periodically to the patches to keep them moist, and infections developed in all participants. Additional groups of men were infected with *Trichophyton mentagrophytes* by applying inocula containing either 1×10^2 or 1×10^4 spores per cm^2 to the forearm or ankle, and occluding the area for 4 d. Infection developed in all subjects. Subsequent to the work of Reinhardt *et al.* (1974), many groups have used a similar protocol and similar numbers of *Trichophyton mentagrophytes* spores to induce nonsystemic infections. The most recent work is that of Aly *et al.* (1991), whose data (relevant to this discussion) are summarized in Table 2-2.

Correspondence Between Experimental and Empirical Data

The experimental data discussed in the preceding pages and summarized in Table 2-2 make it clear that a number of factors contribute to the development of microbial diseases of the skin. These factors include

- The presence on the skin of potentially pathogenic microorganisms;
- A skin microclimate of high temperature and humidity, created experimentally by prolonged periods of occlusion;
- An adequate period of time for pathogenic microorganisms to reach a population size sufficient to initiate infection; and
- An alteration in the integrity of the skin—either by wounding, the action of microbial toxins, or as a consequence of occlusion and hydration of the stratum corneum.

These experimental results also confirm empirical data from Vietnam (Allen, 1989), Colombia (Taplin *et al.*, 1973), Malaya (Sanderson and Sloper, 1953a), the southern United States (Rau *et al.*, 1969), and Panama (Taplin *et al.*, 1965 and Taplin *et al.*, 1967) that show a direct correlation between heat, humidity, skin trauma, and skin disease.

Skin-Surface Microorganisms and Transfer to Internal Mucosa

Staphylococcus aureus is one of several microorganisms that cause toxic shock syndrome and urinary-tract infection (Sande and Mandell, 1985). Its prevalence on the skin of the groin and vulva (Aly and Maibach, 1977; and Aly *et al.*, 1979) suggest that transfer of this microorganism to internal mucosa (e.g., the urethra) may occur and consequently might contribute to the development of these infections. Consequently, a mechanism for removing populations of this microorganism from the skin (e.g., showering or bathing) may reduce the likelihood of its transfer and subsequent contribution to systemic disease. However, such relationships are not clearly defined and cannot be addressed at this time.

Selection of Indicator Microorganisms

Of the five pathogenic microorganisms for which we have data on experimental induction of infection (*Candida albicans*, *Trichophyton mentagrophytes*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*), there are sufficient data only for *Staphylococcus aureus* and *Candida albicans* to model the development of microbial infections of the skin, which is necessary to calculate an optimum showering frequency. It is important to know the average expected population of each of these potentially pathogenic microorganisms on healthy human skin under normal conditions (the baseline population) in order to estimate the time period required for populations of such microorganisms to grow from these background levels to populations similar to those used experimentally as inocula leading to infection. As will be described in the next chapter, we assume that this time period represents an interval during which military personnel are not at risk of developing skin infections.

With regard to baseline population data, we found nothing to indicate that *Trichophyton mentagrophytes* is anything but a transient member of the skin microflora, unable to replicate on the skin under normal conditions (Noble, 1981). The same appears to be true for *Streptococcus pyogenes*, although Elsner and Maibach (1990) found β -hemolytic streptococci (i.e., that group of streptococci, including *Streptococcus pyogenes*, that are human and animal pathogens) on the vulva of female volunteers on day two of their menstruation and on day 21 of their menstrual cycle (but not on day four of menstruation). Aly *et al.* (1982) detected *Pseudomonas aeruginosa* on the skin of individuals studied over a six-month period; approximately 7% of the total study population (n=166) had *Pseudomonas aeruginosa* present on the skin at any given time, although no one consistently carried *Pseudomonas aeruginosa* throughout the entire study period. These data indicate that *Pseudomonas aeruginosa* is also a transient member of the skin microflora, and may not be able to become established on the skin.

Because these organisms do not normally become established on the skin, there are no baseline population data to use to model population growth—consequently, we could not use these species to model the development of microbial infections of the skin. *Trichophyton mentagrophytes* was also not considered further because we had no estimate of the number of spores associated with initiation of infection.

Both *Staphylococcus aureus* and *Candida albicans*, however, can become established on human skin and regularly do so. In fact, Ayliffe *et al.* (1988) observed that the survival of these two microorganisms on intact and unoccluded skin appears

to be better than for other common potential skin pathogens. As noted previously, between 10 and 45% of the human population carries *Staphylococcus aureus* in the nose and/or perineal area (Williams, 1963; and Noble, 1981), and between 3 and 10% of young adults carry *Candida albicans* on the skin (Noble, 1981). Accordingly, we selected these two microorganisms as indicator species to model the development of microbial infections of the skin.

Background Populations of *Staphylococcus aureus* and *Candida albicans*

The number of microorganisms normally present on different skin sites reflects the importance of moisture in microbial growth, with the relatively moist axillae, groin, and toe web supporting populations of microorganisms two to five orders of magnitude greater than those found on dry sites (Marples, 1976; and Aly and Maibach, 1977). We selected baseline population data on *Staphylococcus aureus* and *Candida albicans* from the axilla, groin, toe web, vulva, and perianal (skin around anal opening) area (see Table 2-3). The arithmetic-mean value of the population of each microorganism (per cm² of skin surface area) is used to determine the time period—under specific environmental conditions—necessary for these microorganisms to reach population levels identified in the laboratory as sufficient to initiate infection.

The data on *Candida albicans* (Rebora *et al.*, 1973) and *Staphylococcus aureus* (Singh *et al.*, 1971; and Marples and Kligman, 1972) show that under the favorable conditions of these experiments, both species exhibited logarithmic-phase growth. Consequently, the inoculation population reached a level capable of initiating disease in a relatively brief period. Many factors normally act to prevent such rapid growth of these pathogens (e.g., desiccation, competition from resident flora, removal of microorganisms by washing). In the next chapter, we discuss the efficacy of showering, bathing, and various cleansing substances in removing microorganisms from human skin.

Table 2-3. Baseline populations of *Staphylococcus aureus* and *Candida albicans*.

Microorganism	Anatomical sampling location	Population per cm ² of skin	Comments	Reference
<i>Staphylococcus aureus</i>	Axilla	8.6×10^3	Detection of carriage in 11 healthy male prisoners.	Aly and Maibach (1977)
	Groin	7.3×10^3		
	Toe web	1.6×10^3		
<i>Staphylococcus aureus</i>	Perianal	3.0×10^3	Investigation to compare normal flora of vulva with other anatomical sites among population of 18 healthy women (Caucasian and Filipino, mean age of 39 y).	Aly <i>et al.</i> (1979)
	Vulva	4.1×10^4		
	Arithmetic-mean value	1.2×10^4		
<i>Candida albicans</i>	Axilla	0.07	Detection of carriage in 11 healthy male prisoners.	Aly and Maibach (1977)
	Toe web	3.8		
	Vulva (yeast)	82	Investigation to compare normal flora of vulva with other anatomical sites among population of 18 healthy women (Caucasian and Filipino, mean age of 39 y).	Aly <i>et al.</i> (1979)
	Arithmetic-mean value	28.6		

^a Carriage refers to individuals harboring populations of potentially pathogenic skin microorganisms without manifestation of skin disease.

^b Genus and species of yeast was not specified.

3. Effectiveness of Cleansing in the Prevention of Microbial Infections of the Skin

In this section, we discuss the efficiency of skin cleansing by water alone or in combination with unmedicated soap, medicated soap, or the antiseptic chlorhexidine in reducing or destroying populations of potentially pathogenic microorganisms on human skin. This information is used in Chapter 4 to determine an optimum showering frequency for the prevention of nonsystemic microbial skin infections in military personnel in the field.

Water as a Cleansing Agent

Water has a limited ability to cleanse, and when used alone, will remove only water-soluble matter and whatever insoluble material can be washed off by it. However, the use of running water, such as in a conventional shower, gives more efficient cleaning than simple immersion as a result of the mechanical force of the water stream (Lane and Blank, 1945). In fact, data from experiments conducted by Byrne *et al.* (1990) appear to validate this early observation. The focus of these more recent experiments by Byrne *et al.* (1990) was to assess the efficacy of a 4% chlorhexidine detergent for whole-body disinfection during showering and bathing. Byrne *et al.* (1990) reported a 94% mean reduction in colony counts of total bacteria on the skin of volunteers after a single whole-body shower with water and 50 mL of a 4% chlorhexidine detergent. A single whole-body bath (also with 50 mL of a 4% chlorhexidine detergent) only reduced bacterial colony counts by 71% (Byrne *et al.*, 1990). The fact that the same quantity and concentration of chlorhexidine was used in both the showering and bathing experiments suggests that the greater reduction in bacterial colony counts following a shower is attributable to the mechanical force of the water during showering. Ayliffe *et al.* (1990) have also recognized that skin-surface microorganisms can be removed by a combination of chemical and physical processes when water and a cleansing agent (e.g., medicated or unmedicated soap or detergent) are used simultaneously. Finally, Ojajärvi (1981) found that a single washing with water alone reduced mean counts of *Staphylococcus aureus* and *Pseudomonas spp.* by 77% and 98%, respectively, on the fingertips of volunteers who had been artificially contaminated with these microorganisms.

Water and Unmedicated Soap for Skin Cleansing

For decades, soaps have been used in bathing because of their ability to enhance the removal of dirt, sweat, oils, and microorganisms from the skin (Kooyman and Halberstadt, 1945). Soaps are surface-active agents that, when used in conjunction with water, serve to emulsify both water-soluble and fat-soluble materials on the skin (Sulzberger and Baer, 1945). According to Sulzberger and Baer (1945), once materials on the skin surface have been emulsified in an aqueous-soap solution, rinsing with additional water brings about a constant dilution and removal of the residual soap solution and any other materials remaining in this solution. The efficacy of water and unmedicated soap in removing or destroying potentially pathogenic bacteria from the skin is suggested by data reported by Ojajärvi (1981). These data demonstrated that a single washing with an unmedicated liquid soap removed 83.9% and 98% of *Staphylococcus aureus* and *Pseudomonas spp.*, respectively, from finger tips artificially contaminated with these bacteria. Although water alone or in combination with unmedicated soap appears to disperse populations of skin flora over the body during showering and bathing, such dispersal does not typically lead to skin infection in healthy individuals (Holt, 1971; Davies *et al.*, 1977; and Brandberg and Andersson, 1981).

Water and Medicated Soap for Skin Cleansing

With the recognition that gram-positive bacteria resident in the axilla were involved in the development of body odor (Shelley *et al.*, 1951), antimicrobial ingredients were commonly added to bar soaps to decrease populations of such bacteria (see review by Marzulli and Bruch, 1981). At about the same time, interest developed in whether such soaps could also prevent skin infections by virtue of their antimicrobial activity (i.e., the ability to kill or inhibit growth of bacteria) (see review by Marzulli and Bruch, 1981). In Appendix A, we evaluate published data on the efficacy of antimicrobial soaps and chlorhexidine in the prevention of skin infections. On the basis of that evaluation, we concluded that the antimicrobial soaps currently available as over-the-counter (O-T-C) products, specifically those containing the antimicrobial ingredients triclocarban (TCC; 3,4,4'-trichlorocarbanilide), triclosan [TCS; 5-chloro-2-(2,4-dichlorophenoxy)phenol], and/or cloflucarban [CFL; 4,4'-dichloro-3-(trifluoromethyl)carbanilide], can decrease populations of certain microorganisms on the skin. However, as discussed in Appendix A, whether or not these population decreases are sufficient to prevent microbial diseases of the skin has not been determined unequivocally. Nevertheless, data reported by Roman *et al.* (1957), Finkey

et al. (1984), and Yackovich and Heinze (1985) indicate that repeated washings with a soap containing concentrations of TCC ranging from 0.5% to 2% can reduce populations of gram-positive skin bacteria. In fact, the data of Roman *et al.* (1957) suggest that the magnitude of this reduction increases in direct proportion to the concentration of TCC present in the soap. Furthermore, Roman *et al.* (1957) reported that after repeated handwashings (a minimum of three times per day) of volunteers over a 5-d period and a 12-d period with soap containing 2% TCC, populations of resident skin bacteria were reduced by 96.5% and 97.8%, respectively.

Chlorhexidine—An Antiseptic Skin Cleanser

Chlorhexidine (CHX) is a broad-spectrum topical antibiotic and has been shown to have activity *in vivo* against gram-positive and gram-negative bacteria, and the yeast *Candida albicans* (Beeuwkes, 1958; Davies *et al.*, 1954; and Aly and Maibach, 1976), and *in vitro* against the human immunodeficiency virus (HIV) (Montefiori *et al.*, 1990) (see Appendix A). Data from a clinical trial conducted in Costa Rica (Taplin, 1981) showed that CHX has prophylactic activity against bacterial infections of the skin (see Appendix A). Furthermore, data from the only two whole-body showering experiments reported in the literature, which were designed to examine the removal of skin microorganisms using CHX (Kaiser *et al.*, 1988; and Byrne *et al.*, 1990), indicate that a 4% solution of CHX substantially reduced populations of skin bacteria. For example, Byrne *et al.* (1990) measured a 94% reduction in mean colony counts of total bacteria from the groin and axilla after a single shower; an additional 77.5% reduction was reported after a second shower (assumed to have been taken 24 h after the first shower). Similarly, Kaiser *et al.* (1988) reported a mean reduction of 84.8% in colony counts of staphylococci from the subclavian and inguinal areas 8 to 14 h after a single shower; a second shower, taken several hours after the first, resulted in an additional 88.8% reduction.

Summary

In order to calculate an optimum showering frequency for military personnel in a field setting, data specifically identifying the efficiency with which water, or water in combination with unmedicated soaps, medicated soaps, or CHX could reduce populations of microorganisms on human skin after a single shower are essential. Provision of bathing facilities (e.g., bath tubs) is unlikely in a military setting and data provided by Byrne *et al.* (1990) indicate that bathing is not as efficient as showering in reducing populations of microorganisms on the skin. Unfortunately, efficacy data

(specifically, measurement of populations of microorganisms before and after cleansing) for cleansing with water alone, or water in combination with an unmedicated soap only are available from Ojajärvi (1981); and data regarding cleansing with water in combination with a 4% solution of CHX are only available from Byrne *et al.* (1990) . We use these cleansing efficiencies in conjunction with information on the population dynamics of the indicator microorganisms, *Staphylococcus aureus* and *Candida albicans*, and data on the effect of occlusion and wounding on the growth of skin microorganisms to calculate a permissible interval between showers. This interval should prevent pathogenic microorganisms from reaching populations on the skin capable of initiating infection, assuming that, in general, the efficacy of cleansing with water, or water in combination with an unmedicated soap, or CHX on populations of indicator microorganisms will be the same for all potentially pathogenic microorganisms.

4. Optimum Showering Intervals for Military Personnel in Field Environments

In the preceding chapters, we have identified the principal factors that contribute to the development of microbial infections of the skin:

- The presence on the skin of potentially pathogenic microorganisms (e.g., represented by the indicator species *Staphylococcus aureus* and *Candida albicans*);
- Alteration in the integrity of the skin—either by wounding (including mechanical trauma, minor abrasions such as blisters and chafing, and insect bites), accumulation of cytolytic microbial toxins, or as a consequence of occlusion and the subsequent hydration of the stratum corneum;
- A skin microclimate of high humidity and presumably of high temperature; and
- Time for pathogenic microorganisms to reach a population size that will produce an infection of the skin.

The experimental and empirical data documenting the significance of these factors were also presented. In the absence of such contributing factors, the baseline population of a potentially pathogenic microorganism is not expected to reach a population size sufficient to produce an infection of the skin. We define the “baseline population” to be the population of a potentially pathogenic microorganism that has been observed experimentally to be resident or carried on the skin of individuals without producing infection. The arithmetic-mean values for baseline populations of the indicator organisms, *Staphylococcus aureus* and *Candida albicans*, were calculated in Chapter 2 to be 1.2×10^4 cells/cm² and 2.9×10^1 cells/cm², respectively (see Table 2-3 for derivation). These data are needed to assess the time period required for baseline populations to grow to a size approximately equal to that of the inoculum population used experimentally to induce skin infection. We distinguish an inoculum population from that of a baseline population by virtue of the fact that an inoculum population, while not associated with infection directly, under optimal experimental conditions grows logarithmically to a population threshold observed to be associated with skin infection. Figure 4-1 illustrates the relationship between baseline,

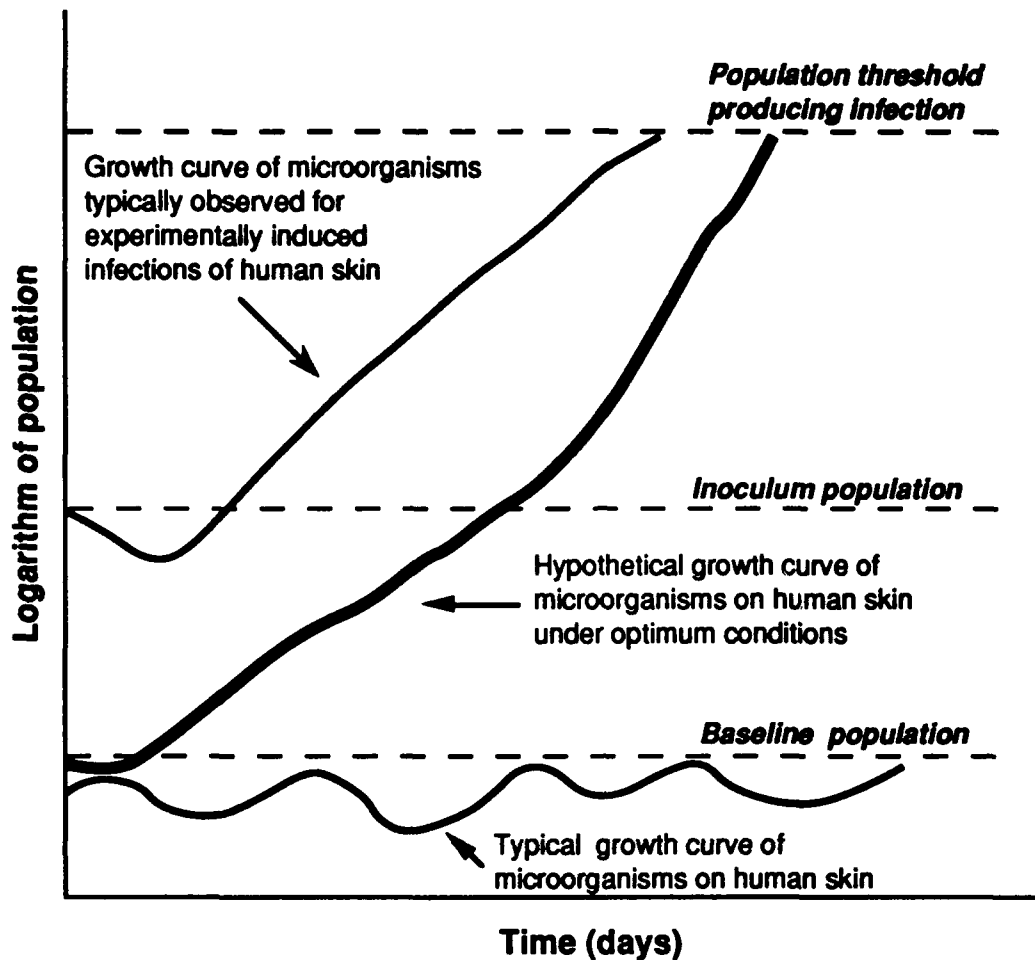


Figure 4-1. Illustration of the relationship between baseline, inoculum, and threshold populations and corresponding hypothetical population dynamics for potentially pathogenic microorganisms.

inoculum, and threshold populations for infection and the corresponding population dynamics for potentially pathogenic microorganisms.

We assume that the time period required for a baseline population to reach that of an inoculum population represents a one-time-only interval in which military personnel do not need to shower. This time interval during which a baseline population grows to a size equal to that of the inoculum population (see Figure 4-1) occurs only when an individual is introduced into a field environment. For purposes of calculating a conservative estimate of this interval, we assume that at the time an individual enters a field environment, conditions will be conducive for the baseline

population to undergo logarithmic-phase growth. Consequently, by the end of the interval the individual is assumed to carry a population of potentially pathogenic microorganisms equivalent to an inoculum population and will be at risk of developing a microbial infection of the skin. Although we will identify this time interval, we do not incorporate it into our recommendation for an optimum showering frequency for military personnel in field environments because it occurs only at the time military personnel are first introduced into a field environment and their subsequent time in that environment cannot be predicted precisely.

Calculation of Showering Frequencies

On the basis of the data presented in Chapter 3, we consider showering to be a mechanism by which microbial populations on the skin can be maintained at or reduced to levels that should not lead to the development of skin infections. Showering appears to reduce microbial populations on the skin by either removing them physically (i.e., by water alone) and/or destroying them chemically (i.e., by use of a soap or an antiseptic) (Ojajärvi, 1981; Kaiser *et al.*, 1988; and Byrne *et al.*, 1990). As noted in Chapter 2, we selected *Staphylococcus aureus* and *Candida albicans* as indicator organisms to model the potential for pathogenic microorganisms to induce nonsystemic microbial skin infection. These indicator microorganisms were selected for the following reasons:

- Data defining baseline population sizes (Aly and Maibach, 1977; and Aly *et al.*, 1979) and describing the population dynamics on human skin are available only for these two potentially pathogenic microorganisms (Singh *et al.*, 1971; Marples and Kligman, 1972; and Reborá *et al.*, 1973);
- Survival of these microorganisms on intact and unoccluded skin appears to be better than for other common, potential skin pathogens (Ayliffe *et al.*, 1988); and
- Epidemiological studies of U.S. military personnel in Vietnam reported by Allen (1989) revealed that these two microorganisms were associated with many debilitating skin infections.

Based on evidence from laboratory experiments (Singh *et al.*, 1971; Marples and Kligman, 1972; Reborá *et al.*, 1973; Reinhardt *et al.*, 1974; Maibach and Kligman, 1962; and Leyden *et al.*, 1980a and 1980b), we conclude that there is a threshold population of

microorganisms (per cm² of skin surface area) that must be achieved before infection is likely to develop. For purposes of developing optimum showering frequencies for military personnel in field environments, we define infection to be the development of an erythematous rash (i.e., redness) with small vesicles or papules (i.e., blisters) such that this condition, if left untreated, may lead to person-days lost from combat for a substantial majority of the population due to performance-degrading infection. The threshold population of *Staphylococcus aureus* and *Candida albicans* (and those pathogenic microorganisms assumed to be represented by these two indicator species) that will cause such infection to develop on the skin occurs as a consequence of logarithmic-phase growth. This logarithmic-phase growth has been induced experimentally by inoculation of these microorganisms on the skin in combination with occlusion, wounding followed by occlusion, or occlusion preceded by the removal of some of the resident flora (Singh *et al.*, 1971; Marples and Kligman, 1972; and Rebora *et al.*, 1973).

The computation of a maximum interval between showers (i.e., an optimum showering frequency) for the reduction or prevention of microbial skin infection in military personnel in field environments is predicated on the following assumptions:

- There is a baseline population of potentially pathogenic microorganisms represented by *Staphylococcus aureus* and *Candida albicans* that can be tolerated on human skin that does not lead to logarithmic-phase growth, unless conditions occur that are similar to those created experimentally by occlusion (see section on Occlusion presented in Chapter 2);
- Microbial infection of the skin from potentially pathogenic microorganisms represented by *Staphylococcus aureus* and *Candida albicans* will not develop unless populations of such microorganisms reach levels that are similar to those that produced infection in laboratory experiments during logarithmic-phase growth (see Rebora *et al.*, 1973; Marples and Kligman, 1972; and Singh *et al.*, 1971);
- Showering (with water or water and a cleansing agent) will prevent the development of skin infection if it can stop a population of potentially pathogenic microorganisms from reaching a size found in laboratory experiments to cause infection;

- Showers will be available to military personnel and will be used in field environments during the time interval in which the indicator microorganisms, *Staphylococcus aureus* and *Candida albicans*, undergo logarithmic-phase growth;
- The frequency of showering will depend on the efficiency with which water or water and a cleansing agent can remove or destroy microorganisms [efficiencies are based on data reported by Ojajärvi (1981) and Byrne *et al.* (1990)]; and
- Military personnel in the field are considered to be healthy males and females, 18 to 55 years old, who have no predisposing physical or mental factors prior to entry into the field environment that would exacerbate health effects.

On the basis of these assumptions, we can mathematically predict the specific showering frequency that will reduce or maintain a population of microorganisms on the skin at a level that should neither produce nor lead to infection.

Consequently, to predict the maximum time interval between showers, we model logarithmic-phase growth according to the following equation (adapted from Stanier *et al.*, 1976):

$$I = [2.303 \times g \times (\log P_{\max} - \log P_{\text{inoc}})] / 0.693, \text{ where} \quad (1)$$

I = time interval between showers (d);

g = generation time for doubling of a microbial population (d);

P_{\max} = maximum tolerable population of microorganisms on human skin (cells/cm²); and

P_{inoc} = the size of the inoculum population on human skin (cells/cm²), under laboratory conditions, when logarithmic-phase growth begins, leading to infection.

The value for P_{\max} is derived by dividing the inoculum population (i.e., P_{inoc}) by the value of one minus the microbial-removal efficiency (i.e., $1 - \eta$) of either a whole-body shower with water or with water and a cleansing agent (i.e., unmedicated or medicated soap or antiseptic). However, our interpretation of the data from laboratory experiments that defined the population threshold associated with

microbial infection of human skin, especially by the indicator microorganisms, *Staphylococcus aureus* or *Candida albicans* (see Table 2-2 and the discussions in Section 2 concerning "Selection of Indicator Microorganisms" and "Background Populations of *Staphylococcus aureus* and *Candida albicans*") indicate that P_{max} cannot exceed P_{inf} , because P_{inf} was the level observed to be associated with infection. Therefore, if the inoculum population should closely approach (e.g., within a factor of 10 or less) the microbial population on human skin at which infection was observed experimentally (P_{inf}), then the derived value for P_{max} may exceed P_{inf} . Should this occur, the value for P_{max} is defined as the microbial population size that is slightly less than that at which infection develops, that is $< P_{inf}$.

The generation time (g) in Eq. 1 is determined from experimental data according to the following mathematical expression (adapted from Stanier *et al.*, 1976):

$$g = [(t_{inf} - t_0) \times 0.693] / [(\log P_{inf} - \log P_{inoc}) \times 2.303], \text{ where} \quad (2)$$

$(t_{inf} - t_0)$ = time interval reported for inoculum population to reach a size associated with infection (d);

t_{inf} = the time at which a population is reached that produces an infection;

t_0 = time at which the inoculum population begins logarithmic-phase growth;

P_{inf} = population threshold on human skin (cells/cm²) at which infection was observed experimentally; and

P_{inoc} = the size of the inoculum population on human skin (cells/cm²), under laboratory conditions, when logarithmic-phase growth begins, leading to infection.

In Table 4-1, we present the generation times derived using Eq. 2 for the indicator organisms *Staphylococcus aureus* and *Candida albicans*, as well as the experimental data from which those values were calculated. Singh *et al.* (1971) induced infection with *Staphylococcus aureus* by applying different populations of inocula to human skin that had first been degermed with alcohol and then occluded after inoculation (we use the arithmetic-mean value from the three different sets of data on *Staphylococcus aureus* listed in Table 4-1). Marples and Kligman (1972) also induced infection with *Staphylococcus aureus* by applying an inoculum population to

Table 4-1. Calculated generation times (g values) for logarithmic-phase growth of *Staphylococcus aureus* and *Candida albicans*, and the data substituted into Eq. 2 to compute these times.

Microorganism (Reference)	Inoculum per cm ² , P _{inoc} (log ₁₀)	Time interval for infection (d)	Infective population per cm ² , P _{inf} (log ₁₀)	Calculated generation time, g (d)
<i>Staphylococcus aureus</i> (Singh <i>et al.</i> , 1971)				
	1.0 × 10 ⁴ (4.00) ^a	3.0	3.0 × 10 ⁶ (6.48) ^a	0.36 ^b
	5.0 × 10 ³ (3.70) ^c	3.0	5.0 × 10 ⁶ (6.70)	0.30 ^b
	1.0 × 10 ² (2.00) ^d	5.0	5.0 × 10 ⁶ (6.70)	0.32 ^b
			Arithmetic-mean value	0.33
<i>Staphylococcus aureus</i> (Marples and Kligman, 1972)				
	4.0 × 10 ³ (3.60)	1.0	1.6 × 10 ⁶ (6.21)	0.12
<i>Candida albicans</i> (Rebora <i>et al.</i> , 1973)				
	2.5 × 10 ³ ^e (3.40)	1.0	2.4 × 10 ⁴ (4.38)	0.31

^a Although a four-day interval is reported by Singh *et al.* (1971) for this experiment as the time during which an inoculum population of 3.7 × 10⁴ cells/cm² reached its infective level of 3.0 × 10⁶ cells/cm², we assume that this infective population actually developed over a three-day period that began at the end of a population decline (to an estimated level of 1.0 × 10⁴ cells/cm²) during the first 24 h after inoculation. This 24-h period of population decline was described by Singh *et al.* (1971) from their study of the population kinetics of *Staphylococcus aureus*. We calculated the magnitude of this population decline during the 24 h after inoculation (from 3.7 × 10⁴ cells/cm² to 1.0 × 10⁴ cells/cm² — a factor of 3.7) as the arithmetic-mean value of the population declines observed by Singh *et al.* (1971) in two different inoculum populations of *Staphylococcus aureus* applied to human skin.

^b These generation times are calculated from the data of Singh *et al.* (1971) based on logarithmic-phase growth that was observed to begin 24-h after inoculation in population kinetic experiments using *Staphylococcus aureus*.

^c The inoculum population originally placed on the skin of the adult volunteers was 1.5 × 10⁴ cells/cm², which then declined to 5.0 × 10³ cells/cm² during the 24 h immediately after inoculation and it is this population that entered logarithmic-phase growth and is considered P_{inoc}.

^d The inoculum population originally placed on the skin of the adult volunteers was 4.0 × 10² cells/cm², which then declined to 1.0 × 10² cells/cm² during the 24 h immediately after inoculation and it is this population that entered logarithmic-phase growth and is considered P_{inoc}.

^e This inoculum population closely approaches the infective population (i.e., within a factor of 10) and leads to a derived value of P_{max} that exceeds P_{inf} (see discussion in text).

human skin that had first been wounded by stripping with tape and then occluded after inoculation. Finally, Rebora *et al.* (1973) induced infection with *Candida albicans* by first applying a population of these organisms (approaching the infective population size) to human skin and then occluding the experimental area. The generation time for *Candida albicans* is based on that data set from Rebora *et al.* (1973) in which infection was observed in 90% of the study population.

An estimate of the time for a baseline population to reach an inoculum population may also be computed using Eq. 1, the generation times presented in Table 4-1 for *Staphylococcus aureus* based on data from Singh *et al.* (1971) and for *Candida albicans* based on data from Rebora *et al.* (1973), and baseline population data for these microorganisms presented in Table 2-3. The generation time calculated for *Staphylococcus aureus* from the data of Marples and Kligman (1972) is not used because these investigators induced infection by wounding human skin and such experimental conditions are not considered relevant for military personnel just entering a field environment. For *Staphylococcus aureus*, the interval of time for a baseline population to reach the inoculum size that led to an experimentally-induced infection was approximately 14 h (0.6 d), and for *Candida albicans* this interval of time was about 48 h (2 d). These estimates are probably conservative because they are based on generation times derived from experiments conducted under optimal growth conditions for these microorganisms. However, as mentioned previously, the time interval for a baseline population to reach a population equivalent to that used as an experimental inoculum, which eventually reaches an infective level, occurs only at the time military personnel are introduced into a field environment. Because the time military personnel might spend in this environment cannot be predicted with any amount of certainty, this interval is not factored into our recommendation for an optimum showering frequency.

Using Eq. 1 with data on removal efficiencies of microorganisms from the skin by water, and by water with unmedicated soap or CHX (see Chapter 3), and the data in Table 4-1 for values of g and P_{inoc} , we calculated the showering intervals that appear in Tables 4-2 through 4-4 for *Staphylococcus aureus* and *Candida albicans*. To prevent development of infection due to *Staphylococcus aureus* on occluded healthy human skin, the showering interval ranges from 0.66 to 1.3 d (see Table 4-2). To prevent development of infection due to *Staphylococcus aureus* on wounded or abraded skin that has been occluded, the showering interval ranges from 0.25 to 0.49 d (see Table 4-3). To prevent development of infection due to *Candida albicans* on occluded

Table 4-2. Calculated showering frequency based on an occlusion experiment (using Saran Wrap), where an inoculum of *Staphylococcus aureus* at 1.0×10^4 cells/cm² (P_{inoc})^a was applied to a forearm degermed with alcohol (Singh *et al.*, 1971).

Experimental conditions	Chlorhexidine	Unmedicated bar soap	Water alone
Removal efficiency of cleanser (%)	94 (Byrne <i>et al.</i> , 1990)	84 (Ojajärvi, 1981)	77 (Ojajärvi, 1981)
Maximum tolerable population of skin microorganisms (P_{max} in cells/cm ²)	1.7×10^5 (based on 94% removal efficiency of cleanser)	6.3×10^4 (based on 84% removal efficiency of cleanser)	4.3×10^4 (based on 77% removal efficiency of cleanser)
Generation time (g; in days) computed directly from experimental data.	0.33 (based on an average of values calculated from Singh <i>et al.</i> , 1971).	0.33 (based on an average of values calculated from Singh <i>et al.</i> , 1971).	0.33 (based on an average of values calculated from Singh <i>et al.</i> , 1971).
Calculated shower interval (d)	1.3	0.88	0.66

^a Chosen because the inoculum population of 3.7×10^4 cells/cm², which we assumed declined to an estimated level of 1.0×10^4 /cm² during the first 24 h after inoculation (and then entered logarithmic-phase growth until reaching the infective level three days later), achieved infection in more adult volunteers (four out of five) than the other inoculum populations utilized by Singh *et al.* (1971) (see Table 4-1).

Table 4-3. Calculated showering frequency based on an occlusion experiment (using Saran Wrap), where an inoculum of *Staphylococcus aureus* at 4.0×10^3 cells/cm² (P_{inoc})^a was applied to a forearm wounded by stripping with tape (Marples and Kligman, 1972).

Experimental conditions	Chlorhexidine	Unmedicated bar soap	Water alone
Removal efficiency of cleanser (%)	94 (Byrne <i>et al.</i> , 1990)	84 (Ojajarvi, 1981)	77 (Ojajarvi, 1981)
Maximum tolerable population of skin micro-organisms (P_{max} in cells/cm ²)	6.7×10^4 (based on 94% removal efficiency of cleanser)	2.5×10^4 (based on 84% removal efficiency of cleanser)	1.7×10^4 (based on 77% removal efficiency of cleanser)
Generation time (g; in days) computed directly from experimental data.	0.12	0.12	0.12
Calculated shower interval (d)	0.49	0.32	0.25

^a The population of 4.0×10^3 cells/cm² was selected for P_{inoc} because it came from the only experiment in which Marples and Kligman (1972) reported the size of both the inoculum and the subsequent infective population.

Table 4-4. Calculated showering frequency based on an occlusion experiment (using Saran Wrap), where an inoculum of *Candida albicans* at 2.5×10^3 cells/cm² (P_{inoc})^a was applied to a forearm (Rebora *et al.*, 1973).

Experimental conditions	Chlorhexidine	Unmedicated bar soap	Water alone
Removal efficiency of cleanser (%)	94 (Byrne <i>et al.</i> , 1990)	84 (Ojajärvi, 1981)	77 (Ojajärvi, 1981)
Maximum tolerable population of skin microorganisms (P_{max} ; in cells/cm ²)	$< 2.4 \times 10^4$ ^b	1.6×10^4 (based on 84% removal efficiency of cleanser)	1.1×10^4 (based on 77% removal efficiency of cleanser)
Generation time (g; in days) computed directly from experimental data.	0.31	0.31	0.31
Calculated shower interval (d)	< 1.0	0.82	0.66

^a The population of 2.5×10^3 cells/cm² was selected for P_{inoc} because this inoculum was below the infective dose and achieved infection in 18 out of 20 adult volunteers.

^b A value of $< 2.4 \times 10^4$ *Candida albicans*/cm² was selected as the value for P_{max} rather than that determined by dividing P_{inoc} by the value of one minus the removal efficiency of the microorganism from the skin by cleansing. This was done because the latter population size exceeded the threshold of 2.4×10^4 *Candida albicans*/cm² that was observed to produce infection on human skin experimentally.

healthy human skin, the showering interval ranges from 0.66 to an interval that is approximately 1 d (see Table 4-4). In all instances the smallest interval is associated with the use of water alone, the greatest interval is associated with the use of water and CHX, and an intermediate interval is associated with the use of water and an unmedicated soap. The showering interval for preventing development of infection by these indicator organisms when water and a medicated soap are used could not be calculated due to the absence of essential data, but this interval would probably approach, but not equal, the interval calculated for CHX.

The predicted intervals between showers shown in Tables 4-2 through 4-4 are based on experimental conditions that were conducive to optimal growth (i.e., occlusion or in the extreme, wounding and occlusion) of the indicator microorganisms. We assume that the microenvironment produced by occlusion (i.e., elevated temperature and humidity) and the resulting hydration of the stratum corneum will be experienced typically by military personnel operating in tropical or semitropical environments. Similar microenvironmental conditions also may develop when occlusive clothing is worn for long periods in temperate, arid, or arctic environments. However, there are no data available in the literature that we can use to model microbial growth and subsequent development of skin infection under less than optimal microenvironmental conditions. Furthermore, in the absence of specific data about the population dynamics and etiology of skin disease produced by pathogenic microorganisms other than *Staphylococcus aureus* and *Candida albicans*, we must assume that the showering intervals calculated for those indicator microorganisms also should reduce or prevent disease caused by other pathogens.

Recommendations for Showering Intervals

On the basis of the limited nature of the data available for (1) estimating the efficiency of removal of skin microorganisms by whole-body showering with either water alone or water and a cleansing agent (i.e., unmedicated soap, medicated soap, or chlorhexidine antiseptic); (2) determining the inoculum population of potentially pathogenic microorganisms that will undergo logarithmic-phase growth to reach a population size that can produce a skin infection; (3) deducing the precise population of potentially pathogenic microorganisms that will produce a skin infection; and (4) validating the relationship between the population dynamics of the indicator microorganisms we selected and the population dynamics of all potentially pathogenic microorganisms, there is a great deal of uncertainty associated with the ranges of values for showering intervals mentioned above and appearing in Tables 4-2

through 4-4. Nonetheless, each showering interval was determined on the basis of growth dynamics for the indicator microorganisms, *Staphylococcus aureus* and *Candida albicans*, that occurred under optimal conditions (i.e., occlusion or in the extreme, wounding and occlusion) in a laboratory. As noted previously in this report, such optimal conditions for growth are most likely to be duplicated in a tropical environment. Consequently, without additional data, the showering intervals that are estimated in Tables 4-2 through 4-4 are probably the shortest ones that can be considered at this time for reducing or preventing microbial infections of the skin in military personnel in field environments. On the basis of the calculated showering intervals (Tables 4-2 through 4-4), the following practical recommendations are made:

- To prevent a microbial infection from developing on occluded but relatively healthy human skin (i.e., intact) the interval between showers for military populations deployed in tropical environments (or in environments where the periodic combination of temperature, humidity, and precipitation may approximate that of the tropics) should not exceed (1) 24 h (exactly 1 d), if chlorhexidine antiseptic is used with water (or slightly less than 24 h, if a medicated soap is used with water); (2) 19 h (for practical purposes, 1 d), if unmedicated soap is used with water; and (3) 15 h (about 0.5 d), if only water is used;
- To prevent a microbial infection from developing on occluded and wounded, abraded, or otherwise damaged human skin, the interval between showers for military populations deployed in tropical environments (or in environments where the periodic combination of temperature, humidity, and precipitation may approximate that of the tropics) should not exceed (1) approximately 12 h (about 0.5 d), if chlorhexidine antiseptic is used with water (or slightly less than 12 h if a medicated soap is used with water); (2) about 7.5 h (virtually 0.3 d), if unmedicated soap is used with water; and (3) 6 h (exactly 0.25 d), if only water is used; and

- Longer intervals between showers than those recommended above for preventing a microbial infection on human skin probably are acceptable, if environmental and microenvironmental conditions for stimulating microorganisms to enter logarithmic-phase growth are not optimum. However, the maximum interval between showers should never exceed 7 d, whether or not water is used alone or with a cleansing agent, as a 7-d interval should prevent infestations of the body louse and subsequent spread of louse-borne disease, as well as provide a positive effect on morale.

The range of showering intervals identified above for reducing or preventing microbial skin infection in military personnel are either determined from the results of limited experiments involving inoculation of the skin with microorganisms (i.e., the indicator microorganisms *Staphylococcus aureus* and *Candida albicans*) followed by complete occlusion with Saran Wrap, or are based on the disruption of the life cycle of the body louse. Until more complete information is available concerning relationships between (1) environmental conditions (e.g., temperate, arctic, or desert climates that are geographical and/or seasonal), (2) fabric coverings with occlusive properties different from Saran Wrap, (3) the efficacy of cleansing agents, and (4) the relative health of the skin, a more accurate range of showering intervals that is applicable to each specific environment that may be encountered by U.S. military personnel cannot be provided.

5. Conclusions and Research Recommendations

We have used the limited data on the development of microbial infections of the skin and on the prevention of such infections by showering with water or water and a cleansing agent to calculate showering frequencies that should prevent nonsystemic microbial infections of the skin in military personnel. Although there is uncertainty associated with our recommendations, there is a strong element of conservatism incorporated into each one (e.g., we used data from laboratory studies where infection was experimentally induced only under optimal conditions such as occlusion or wounding and occlusion). Our analyses indicate that adherence to a specific showering frequency can be an effective mechanism for preventing skin disease, but that the interval of time that can elapse between showers will depend on the cleansing agent that is used. For example, the largest acceptable time interval between showers is associated with showering with water and a 4%-chlorhexidine antiseptic solution. It is presumed that the interval between showers with water and a medicated soap would be only slightly less than that interval between showers with water and chlorhexidine. The time intervals between showers if only water or water and an unmedicated soap are used are predicted to be much less than that interval associated with the use of water and chlorhexidine. Data reported by Byrne *et al.*, (1990) indicate that more than one shower with water and chlorhexidine should maintain populations of potentially pathogenic microorganisms at relatively low levels between showers; however, there is no clear evidence that this is true for other cleansing agents. Thus, microbial populations are more likely to recover in size between showers with water or water alone than between showers with water and chlorhexidine. Consequently, if water alone or water and medicated or unmedicated soap are used in showering, then it is important that the recommended showering frequency be strictly observed.

We also conclude that under conditions where skin has been wounded and occluded, an interval between showers of much less than one day may need to be adopted, no matter what cleansing agent is used. For example, showering intervals ranging from a maximum of 0.5 d to a minimum of 0.25 d are estimated for showering with water and chlorhexidine or for showering with water alone under such circumstances (see Table 4-3). Furthermore, there are no data available to calculate an interval between showers for individuals under conditions that are not conducive to optimal microbial growth. Consequently, under such conditions (e.g., nonocclusive clothing worn intermittently in a temperate climate), we recommend that the U.S. Army continue to provide showers at least once every 7 d. The basis for this

recommendation is that showering every 7 d will disrupt the life cycle of the body louse, which can cause outbreaks of pediculosis—a highly contagious condition which can result in a maculopapular rash, pruritis, and pyoderma (Busvine, 1985). Also, showering at least every 7 d should have a positive effect on morale. Finally, it is impossible from available data to predict the role that sex, age, or race may play in the development and manifestation of microbial-induced skin infections for military personnel in field environments. Thus, the intervals between showers that we have recommended are assumed to be applicable to all military personnel.

Research Recommendations

The paucity of data on the relationship between hygiene and microbial skin infections makes it impossible to verify or validate the assumptions and subsequent calculations we used to derive recommended maximum showering frequencies for U.S. military personnel in field environments. Therefore, we urge that the following research be performed to achieve such verification and validation and reduce the uncertainty in our recommendations:

- Relevant information should be obtained and evaluated from “after-action” reports from recent military conflicts in Grenada, Panama, and the Persian Gulf (which took place during the 1980s and early 1990s);
- Field experiments should be performed with military personnel to determine microbial populations on the skin associated with the wearing of different fabrics in different climates;
- Laboratory experiments should be conducted to determine if occlusion alone (i.e., without inoculation and wounding) can produce microbial infections of the skin and, if such infections are produced, what microorganisms typically produce these infections under such conditions;
- Investigations should be conducted to determine the period of antimicrobial activity of medicated soaps under normal conditions in a temperate climate and how such activity is affected by different climates and microclimates;
- Studies should be performed to obtain more accurate data on the population dynamics of skin microorganisms before and after showering with water and water and cleansing agents or after using antimicrobial towelettes under circumstances where showering may not be feasible;

— Experiments should be conducted to compare and contrast the effects of chlorhexidine, antimicrobial soaps, unmedicated soaps, and water on the microbial flora of human skin under conditions of occlusion, particularly by fabrics used in military uniforms worn in field environments.

— A determination should be made of the period of time that soil, sweat, feces, or urine can remain in contact with human skin without adverse effect; how that period of time may vary with climate and different levels of physical activity; and how the presence of these substances on human skin may contribute to the development of microbial skin infections.

— Laboratory studies should be performed to determine how race, gender, climate, the microenvironment produced by clothing, and different showering frequencies affect populations of resident microorganisms on the skin and their relationship to induction of skin infections.

Additional specific research recommendations for medicated (i.e., antimicrobial) soaps and chlorhexidine antiseptic are presented at the end of Appendix A.

The recommendations made in this report can only be improved or validated if additional research is performed. Additionally, acquisition of new data is essential for the U.S. Army to accurately determine the appropriate showering protocol and to identify the most effective and practical cleansing agent to ensure that combat effectiveness is not impaired by microbial infections of the skin.

6. References

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Appendix A

Effectiveness of Antimicrobial Soaps and Chlorhexidine in the Prevention of Skin Infections

Introduction

For soldiers in a combat setting, morbidity from diseases of the skin has caused significant losses of manpower. Among U.S. military combat troops in Vietnam, for example, disability from skin disease was one of the single most important medical causes of man-days lost from combat (Blank *et al.*, 1969; and Allen, 1989).

In this Appendix, we evaluate whether the use of any antimicrobial soap or similar product can decrease the population of disease-causing microbes on the skin, and by so doing, decrease the incidence of skin infections that might cause man-days lost from combat.

Active Ingredients of Antimicrobial Bar Soaps

Hexachlorophene, HCP [2,2'-methylenebis(3,4,6-trichlorophenol)] was developed in the 1930's as an antimicrobial ingredient for topical application (see review by Gump, 1969). By the 1960's, use of HCP was widespread, and it was a common and effective ingredient in a number of over-the-counter (O-T-C) antimicrobial preparations (see reviews by Marzulli and Bruch, 1981; and Eiermann, 1981). Evidence accumulated, however, that clearly demonstrated the systemic toxicity of small amounts of topically applied HCP, especially to human infants (e.g., Kimbrough and Gaines, 1971; Curley *et al.*, 1971; and FDA, 1972).

In response to health and safety concerns that surfaced regarding HCP, the Food and Drug Administration (FDA) in the U.S. Department of Health, Education, and Welfare, convened the Over-the-Counter (O-T-C) Antimicrobial I Drug Review Panel (hereafter referred to as the Panel) in 1972 to review manufacturer's claims regarding O-T-C antimicrobial products. From 1972 to 1978, the Panel reviewed product claims and the safety and efficacy of antimicrobial ingredients formulated and sold for a variety of purposes [e.g., "skin antiseptic," "surgical hand scrub," and "health-care personnel hand wash," (as defined in FDA (1974)]. During this period the Panel analyzed safety and efficacy data for HCP, various halogenated salicylanilides, and certain carbanilides, all of which were common ingredients of antimicrobial soaps at the time of the Panel review.

Table A-1 lists the common names and acronyms, chemical names, and regulatory status of the compounds addressed by the FDA. The findings of the panel with regard to each of these compounds are described next.

Hexachlorophene

The Panel reviewed data on the toxicity of HCP to humans, and on the basis of this information, recommended that the FDA remove HCP from O-T-C use (FDA, 1972). Accordingly, the FDA issued a Final Order that made HCP available only by prescription, except when added to products as a preservative at a concentration of < 0.1% (FDA, 1972). The FDA action specifically prohibited the use of HCP for " ... routine prophylactic total body bathing ... "—a finding that effectively eliminated the use of HCP in O-T-C antimicrobial soaps.

Halogenated Salicylanilides

The halogenated salicylanilides constitute a class of compounds that were commonly added to bar soaps in the 1960's and 1970's for their antimicrobial activity. The substantivity (i.e., the persistence of the chemical on the skin after rinsing) and water-insolubility of the salicylanilides, particularly 3,4',5-tribromosalicylanilide (tribromsalan, TBS); 4',5-dibromosalicylanilide (dibromsalan, DBS); 3',5-dibromosalicylanilide (metabromsalan, MBS); 3,3',4,5'-tetrachlorosalicylanilide; and 3,5-dibromo-3'-trifluoromethylsalicylanilide (fluorosalan) apparently made them ideal ingredients for bar soaps (see reviews by Marzulli and Bruch, 1981; and Eiermann, 1981).

These halogenated salicylanilides were the subject of intense scrutiny by the Panel because of reports that they caused persistent and sometimes disabling photosensitization (i.e., an abnormal sensitivity to sunlight that results in severe dermatitis) (FDA, 1974). The Panel found the evidence on photosensitization convincing, and in 1975 a regulation was published in the *Federal Register* (FDA, 1975) that stated in part " ... halogenated salicylanilides are not generally recognized as safe and effective for use as active or inactive ingredients in any drug products."

The net effect of the regulatory actions against HCP and the halogenated salicylanilides by the FDA was to remove from the market effective antimicrobial substances that might serve as ingredients of soaps.

Table A-1. Active ingredients of antimicrobial soaps.

Common name	Assigned acronym	Chemical name	Regulatory status ^a
Hexachlorophene	HCP	2,2'-methylenebis(3,4,6-trichlorophenol)	(1)
Halogenated Salicylanilides:			
Tribromsalan	TBS	3,4',5-tribromosalicylanilide	(2)
Dibromsalan	DBS	4',5-dibromosalicylanilide	(2)
Metabromsalan	MBS	3',5-dibromosalicylanilide	(2)
NA ^b	None	3,3',4,5'-tetrachlorosalicylanilide	(2)
Fluorosalan	None	3,5-dibromo-3'-trifluoromethylsalicylanilide	(2)
Carbanilides:			
Triclocarban	TCC	3,4,4'-trichlorocarbanilide	(3)
Cloflucarban	CFL	4,4'-dichloro-3-(trifluoromethyl)carbanilide	(3)
Triclosan	TCS	5-chloro-2-(2,4-dichlorophenoxy)phenol	(3)
Other Compounds:			
Phenol \leq 1.5%	None	Phenol	(3)
PCMX	None	Para-chloro-meta-xyleneol	(3)

^aRegulatory status is defined by the numerical values 1, 2, or 3, where
 (1)Hexachlorophene was banned from O-T-C use by order of the FDA (1972);
 (2)Tribromsalan, Dibromsalan, Metabromsalan, Fluorosalan, and 3,3',4,5'-tetrachlorosalicylanilide were removed from the market in 1975 (FDA, 1975); and
 (3)A final determination of the regulatory status of Triclocarban, Cloflucarban, Triclosan, "phenol \leq 1.5%", and PCMX has not been made (see FDA, 1978).

^bNA = not available.

Carbanilides

The Panel (FDA, 1974 and 1978) also raised questions about the safety and efficacy of certain carbanilides, specifically 3,4,4'-trichlorocarbanilide (triclocarban, TCC), 4,4'-dichloro-3-(trifluoromethyl)carbanilide (cloflucarban, CFL), and a halogenated diphenyl ether, 5-chloro-2-(2,4-dichlorophen-oxy)phenol (triclosan, TCS). In the FDA's Tentative Final Order (FDA, 1978), the Commissioner of the FDA concluded that data on the safety and efficacy of these substances were not

sufficient to make a final determination. This Tentative Final Order included provisions that permitted these substances to remain in O-T-C antimicrobial soaps and other products for two years following issuance of a Final Order, provided tests and studies were conducted to satisfy concerns raised by the Panel about safety and efficacy. However, the findings of the Panel were never incorporated into a final form, and the regulatory status of CFL, TCS, and TCC for use as antimicrobial ingredients is unclear.

Other Compounds

In addition to the compounds already noted, the Panel briefly considered "phenol 1.5% or less in aqueous/alcoholic solution" (phenol \leq 1.5%) and para-chloro-meta-xylene (PCMX) as antimicrobial ingredients of soaps (FDA, 1974 and 1978). Many questions on the safety and efficacy of these two compounds were raised by the Panel.

For phenol \leq 1.5%, the concerns centered on the profoundly toxic effects of this substance at concentrations greater than 1.5%, and the fact that no data were presented to indicate that phenol at a concentration of \leq 1.5% was an effective antimicrobial agent. This substance has never been evaluated in a clinical trial, either singly, or in combination with other antimicrobial ingredients, and is apparently no longer marketed as an ingredient of O-T-C antimicrobial soaps.

According to the Panel, almost no data were submitted on the safety or efficacy of PCMX (FDA, 1974 and 1978). The Panel (FDA, 1974 and 1978) concluded that "There are so little data available that it is the view of the Panel, that this ingredient should be tested as if it were a new chemical entity for use in antimicrobial formulation(s)." PCMX is currently available in certain liquid soaps (Keswick, 1991). However, we were unable to find any citations in the open scientific literature that indicated comprehensive safety and toxicity tests were conducted on PCMX. Because of the extremely limited information available on phenol \leq 1.5% and PCMX, we did not consider either substance as a candidate for use by U.S. military personnel.

Clinical Trials of Antimicrobial Bar Soaps

There is a long history of interest in the potential of soaps that contain antimicrobial ingredients to prevent or minimize the incidence of skin disease. Beginning in the 1960's, several pharmaceutical companies initiated clinical trials of bar soaps that contained antimicrobial ingredients with the hope of establishing the

effectiveness of these products in preventing nonsystemic skin infections (Leonard, 1967; Dubow and Winter, 1967; Duncan *et al.*, 1969; MacKenzie, 1970; Sharrett *et al.*, 1974; and Taplin, 1981 *Arizona study*). Details of these studies are summarized in Table A-2.

With the exception of Sharrett *et al.* (1974), who studied children in rural Trinidad, the subjects of each study were drawn from highly restricted populations. Study participants typically lived in an institution: military academies (Leonard, 1967 and MacKenzie, 1970), prison farms (Duncan *et al.*, 1969), a boarding school (Taplin, 1981 *Arizona study*), or a juvenile detention home (Dubow and Winter, 1967). Within each study population, subjects ate similar food, had similar activities, and usually had similar (if not identical) hygiene regimens. These factors provided some uniformity to the study populations, which were not specifically matched with respect to race, age, or socioeconomic status.

Each of the six studies summarized in Table A-2 was designed to examine the efficacy of an antimicrobial bar soap commercially available at the time of the study. There was little similarity in the specific formulations of the soaps used, but all contained a combination of two or more of the following active ingredients: HCP, TBS, DBS, MBS, TCC, CFL. As already noted, HCP is no longer an ingredient of antimicrobial soaps available O-T-C (FDA, 1972); TBS, DBS, and MBS were removed from the market entirely (FDA, 1975); and questions concerning the safety and efficacy of CFL apparently led manufacturers to voluntarily remove it from antimicrobial soaps marketed O-T-C (FDA, 1974 and 1978). Only TCC is still added to antimicrobial bar soaps today, although CFL is theoretically available as well.

Because none of the specific combinations of antimicrobial ingredients contained in the soap bars in the studies cited above are still available, the data and conclusions concerning the efficacy of a specific antimicrobial soap cannot be extrapolated to the antimicrobial soaps currently sold O-T-C. Nonetheless, it is of some interest to briefly review the studies listed above—in part because CFL and/or TCC were ingredients in all of the antimicrobial bar soaps studied. Additionally, reviewing these studies provides some perspective on how questions concerning the efficacy of antimicrobial soaps have been addressed experimentally. Data from these studies also make it clear that even frequent showering (e.g., up to three times a day) using either a plain soap (i.e., no antimicrobial ingredients) or an antimicrobial soap will not eliminate completely skin infections from a population,

Table A-2. Clinical trials that examined the efficacy of antimicrobial soaps and chlorhexidine in preventing nonsystemic bacterial skin infections.

Study	Cleanser	Active ingredient(s)	Study duration	Shower/bath frequency	Supervised	Population	Conclusion
Leonard, 1967 (U.S. Military Academy West Point, NY)	Antimicrobial (bar) soap	2% mix of dibromsalan, tribromsalan, triclocarban, and cloflucarban	2 mo	Not mentioned	Yes	474 treated 609 control (New Cadets)	Positive effect
Dubow and Winter, 1967 (juvenile detention home, NY)	Antimicrobial (bar) soap	0.75% hexachlorophene and 0.75% triclocarban	Not mentioned	Bathed daily	Yes	160 treated 160 control (8 to 16 y olds)	Effective for moderate to severe wounds
Duncan et al., 1969 (Prison farm(s), TX)	Antimicrobial (bar) soap	2% mix of tribromsalan, triclocarban, and cloflucarban	9 mo	Shower 1x/d or ad libitum	Yes No	2550 w/crossover 1275 treated/control (Adults)	Equivocal results, yet positive effect reported for 1x/d
MacKenzie, 1970 (U.S. Naval Academy, Annapolis, MD)	Antimicrobial (bar) soap	0.75% hexachlorophene and 0.75% triclocarban	6 mo	Shower 3x/d	Yes	602 treated 599 control (1st yr midshipmen)	Positive effect
Sharnett et al., 1974 (Homes, Trinidad)	Antimicrobial (bar) soap	0.75% hexachlorophene and 0.75% triclocarban	2 mo	Wash lesions and legs 2x/d	Instructions to parents	135 patients (≤13 y of age)	Not effective
Taplin, 1981 (Navajo Boarding School, AZ)	Antimicrobial (bar) soap	1% triclocarban and 0.5% cloflucarban	2 mo	Shower 1x/d	Yes	322 treated 311 control (7 to 12 y olds)	No effect on skin infection
Taplin, 1981 (Schools, Costa Rica)	Antiseptic (cationic) liquid spray	2% chlorhexidine gluconate in distilled water with 4% isopropanol	6 wk	Sprayed from neck to feet, excluding genitalia and buttocks (Mon to Fri only)	Yes	151 treated 153 control (9 y olds)	Effective for reducing skin infection

although such cleansing may reduce their incidence. Finally, these studies provide an historical context for a study described by Taplin (1981 *Costa Rica study*) in which a team of investigators from the University of Miami and the University of Costa Rica were able to demonstrate that a solution of 1:6-di-4'-chlorphenyldiguanidohexane (chlorhexidine, CHX), an antiseptic, was effective in preventing skin infections in a population, when sprayed on the skin daily.

Clinical Trials Reviewed by the Food and Drug Administration

At the time the Panel was convened (see FDA, 1972), there was a great deal of interest in whether the regular use of antimicrobial soaps would prevent nonsystemic microbial infections of the skin. Accordingly, the Panel heard testimony from scientists active in this area of research, reviewed submissions from various pharmaceutical companies, and evaluated the results of Leonard (1967), Duncan *et al.* (1969), and MacKenzie (1970) (see Table A-2). A summary of the Panel's comments follows our discussion of the study by MacKenzie (1970).

The first of these studies (Leonard, 1967) was designed to evaluate the efficacy of an antibacterial bar soap that contained a 2% mixture of TBS, DBS, TCC, and CFL. Study participants consisted of 1083 new cadets at West Point Military Academy in New York. A double-blind technique was used in assigning companies of cadets to either the control group (plain soap) or treatment group (antimicrobial soap). Over the course of the nine-week study, all suspected skin infections were reported to medical investigators for diagnosis and treatment. In some, but not all instances, infections were reportedly verified by culture (the results of these cultures were not published).

When Leonard (1967) evaluated the overall incidence of infections for the entire study period, he found that there were significantly ($p < 0.05$) fewer infections of the skin among those men who used the antimicrobial soap compared to the control group. However, when the incidence of skin infection was evaluated on a weekly basis, the men in the group that used the antimicrobial soap had fewer infections in only six of the nine weeks of the study. Furthermore, the baseline incidence of skin infections among the two study groups was not tabulated, making it difficult to evaluate the absolute significance of the apparent effect of the antimicrobial soap. In addition, not all infections were verified by microbiological cultures, and more than one doctor made the diagnoses of infections. Finally, Leonard (1967) neglected to state how often cadets showered. Consequently, it is possible that some of the reported differences in the incidence of skin infection

between the two groups could be attributed to differences in showering frequency and not to any antibacterial activity of the soap.

Duncan *et al.* (1969) selected the populations of two prison farms in Texas to study the antimicrobial activity of a bar soap that contained a 2% mixture of TBS, TCC, and CFL. A total of 2550 men participated in this study. The prison farms reportedly had similar living conditions, bathing facilities, and work requirements. Each of the two farms had two separate housing units. This arrangement resulted in four separate population groups, which were designated by the investigators as either R1, R2, C1, or C2. Soap was assigned to the groups by a double-blind technique. At the half-way point of the 37-week study, the type of soap each group was using was exchanged for the alternate type. All inmates were examined periodically without prior notification to determine the overall reliability of reporting. All skin lesions were reported to the prison medical department, and any men who reported a skin lesion were examined weekly thereafter. No undocumented skin lesions were found. One of the potentially confounding factors of this study is that showering frequency differed between the units. In the groups designated R1 and C1, the men were required to shower daily Monday through Friday. In the groups designated R2 and C2, the men had showering facilities available seven days a week. However, the men in R2 and C2 were not required to shower at any specific interval, and Duncan *et al.* (1969) did not attempt to document the actual frequency of showering. In groups R1 and C1, Duncan *et al.* (1969) observed a statistically significant decrease ($p < 0.01$ and $p < 0.05$, respectively) in the incidence of skin infections during the period the antimicrobial soap was used. No decrease in the incidence of skin infections was documented in group R2 during use of the antimicrobial soap, and a nonsignificant decrease in the incidence of infection was observed in group C2 during their use of the antimicrobial soap. One of the inferences that can be made from these data is that the antimicrobial soap in question had to be applied with great regularity to be effective. If this presumption is correct, it raises questions concerning the substantivity of the active ingredients—at least under the adverse physical and environmental conditions of a prison work farm in Texas.

MacKenzie (1970) evaluated the effectiveness of an antimicrobial soap that contained 0.75% HCP and 0.75% TCC among first-year midshipmen at the U.S. Naval Academy, Annapolis, MD. The 1201 study participants were divided into two groups, so that approximately half of the men used the antimicrobial soap and the other half used plain soap. MacKenzie (1970) did not use a double-blind technique

in assigning soap. One of several doctors recorded all skin infections reported by participants, and although MacKenzie (1970) did not generally make the initial diagnosis of infection, he was able to observe virtually all cases. Approximately 75% of the infections were cultured and the majority of these cultures yielded *Staphylococcus* spp. Midshipmen were able to shower as often as desired, and MacKenzie (1970) noted that "... three or more showers a day were routine." Over the six-month study period, 41 of the 599 men in the control group (6.8 %) and 23 of the 602 men that used the antimicrobial soap (3.8 %) developed bacterial infections of the skin. One of the men in the antimicrobial-soap group, and three men who used plain soap had a recurrence of the original infection. The difference in infection incidence between the two groups was statistically significant ($p < 0.01$).

The Panel's review (FDA, 1974) of Leonard (1967) acknowledged that there was an apparently significant difference in the incidence of skin infections among the two groups of men. They noted, however, that although the incidence of single furuncles (i.e., boils) was significantly higher in the control group, the incidence of multiple furuncles was not markedly different between the two groups. With respect to MacKenzie's study at Annapolis (MacKenzie, 1970), the Panel cited a lower incidence of cellulitis (i.e., an inflammation of subcutaneous tissue) secondary to trauma, as well as a lower incidence of pustular folliculitis in the group of men that used the antimicrobial soap (FDA, 1974). The Panel also observed that the incidence of secondarily infected skin lacerations was the same in the control and treated groups. Although the Panel did not make specific criticisms of the study reported by Duncan *et al.* (1969), the written comments of Dr. Clarence Livingood apparently stated the consensus of opinion regarding all three studies. Dr. Livingood determined that the results of all three studies were equivocal, and explained this conclusion in part by the following factors:

- (1) There was variation in diagnosis resulting from the use of more than one physician for diagnosis.
- (2) It seemed probable that a 2 [sic] month study was too short to establish the expected results.
- (3) The results of the bacteriologic cultures were not included.
- (4) The initial infection rate was not uniform in the test groups.

However, with regard to the studies of MacKenzie (1970) and Duncan *et al.* (1969), Marples (1971) observed that the study populations exhibited relatively low rates of spontaneous infection (i.e., baseline-infection rates), thereby making it difficult to demonstrate the efficacy of the antimicrobial preparations. Nonetheless, Marples (1971) concluded that "... these studies did show that such soaps significantly lessened the incidence of infection."

Antimicrobial soaps and the treatment of Erythrasma. Erythrasma is a chronic bacterial infection of the skin caused by *Corynebacterium minutissimum* (Sarkany *et al.*, 1962). Although various systemic antibiotics are available to cure this disease, several studies were conducted in the 1960's to evaluate whether use of an antimicrobial soap (without concurrent oral antibiotic therapy) could be an effective treatment (Kooistra, 1965; Dodge *et al.*, 1968; and Taber *et al.*, 1969).

Study results showed unequivocally that daily use of an antimicrobial soap that contained either 0.75% HCP and 0.75% TCC (Taber *et al.*, 1969) or a 2% mixture of DBS, TBS, TCC, and CFL (Kooistra, 1965 and Dodge *et al.*, 1968) resolved erythrasma in the majority of cases. The Panel (FDA, 1974) concluded that the data contained in these three studies constituted definitive evidence of the therapeutic effectiveness of antimicrobial-containing soaps against erythrasma. However, the Panel (FDA, 1974) also determined that conclusions from these data could not be extrapolated to other disease-causing microbes of the skin.

Studies of Antimicrobial Soaps Not Reviewed By The Panel

Reports of three clinical trials of antimicrobial bar soaps were not evaluated by the Panel (FDA, 1974 and 1978). These three clinical trials were conducted by Dubow and Winter (1967), Sharrett *et al.* (1974), and Taplin (1981 *Arizona study*).

Dubow and Winter (1967) initiated a study to compare the frequency of wound infection among boys (ages 8 to 16) who used either plain soap or an antimicrobial soap. The 320 study participants were housed in eight dormitories at a juvenile-detention facility in New York. Half of the dormitories were assigned plain soap, the other half were given an antimicrobial soap that contained 0.75% HCP and 0.75% TCC. Until the final tabulation of data was complete, neither the investigators nor the subjects knew which type of soap each group of boys had received. Daily supervised bathing with the assigned soap was required of all participants. Dubow and Winter (1967) did not specify whether "bathing" meant immersion in a bath tub or showering. Whenever a boy received a cut or abrasion,

it was washed by a nurse with the subject's assigned soap. For purposes of data analysis, these wounds were classified as mild, moderate, or severe.

Among subjects that suffered mild wounds, there was no statistically significant difference in the frequency of infection between those who bathed with the antimicrobial soap and those who used the plain soap. The incidence of infection among individuals who received either moderate or severe wounds was evaluated as a single data set. Dubow and Winter (1967) found a significantly ($p < 0.01$) lower incidence of infection among those participants that used the antimicrobial soap. They also observed that the "overall healing" of wounds was significantly ($p < 0.01$) better in those same individuals. However, Dubow and Winter (1967) failed to specify the duration of the study, whether one or several doctors were involved in the diagnosis of infection, or what the baseline infection rate was among the study participants. These factors, and the highly subjective nature of much of the data in this study, make it impossible to draw any conclusions regarding the efficacy of the antimicrobial soap that was used.

Sharrett *et al.* (1974) selected rural Trinidad as a study location because this geographic region is subject to recurrent epidemics of acute glomerulonephritis associated with *Streptococcal* skin infections. Seventeen families of school-age children were chosen at random from two different villages, and 135 children eventually participated in the study. Neither village had running water and each was dependent on irregular truck deliveries for all of their water needs. In the first eight-week phase of the study, parents were given plain soap and were instructed to wash their children's legs and any skin lesions twice daily. During a second eight-week period the same procedure was followed, but a soap containing 0.75% HCP and 0.75% TCC was used. Finally, after a rest period of two weeks, children were given two separate penicillin injections—one at the beginning and one at the end of a four-week interval. To the disappointment of Sharrett and his coworkers, neither plain soap or the antimicrobial soap significantly decreased the incidence of skin lesions or the prevalence of *Streptococci* in the lesions. However, skin lesions were "somewhat less likely" to harbor *Streptococci* during the period of use of the antimicrobial soap. Not surprisingly, the presence of *Streptococci* decreased to zero following each injection of penicillin.

As described by Sharrett *et al.* (1974), both of the Trinidad study sites were on the banks of a river where mosquitoes are present throughout the year. All study subjects were poor children who went barefoot and barelegged, thereby increasing the likelihood of insect bites and other skin trauma. These factors alone presented

formidable problems to investigators. In addition, it was not possible to supervise (and thus verify) the frequency and thoroughness with which the children's legs were washed. Finally, because whole-body bathing was not practiced due to a lack of water, there was ample opportunity for *Streptococci* to be transferred from unwashed parts of the subject's bodies to their skin lesions. The authors noted that this study was a trial of *soap use* in rural Trinidad rather than simply a trial of soap, and as such, served to point out the obstacles involved in reducing the incidence of skin disease under adverse conditions.

The first study described by Taplin was conducted in a Navajo boarding school in Arizona (Taplin, 1981 *Arizona study*). All 633 children attending the school (ages 7 to 12) participated in this study, and participants were divided into two groups of approximately equal size. One group was assigned a soap that contained 1.0% TCC and 0.5% CFL, the other group was given a plain (i.e., nonmedicated) soap to use. At the beginning of the two-month study, all children were examined by two dermatologists, who documented the number of skin infections. These infections were subsequently cultured on selective media for *Staphylococcus aureus* and *Streptococcus pyogenes*. The baseline incidence of infection was similar in the two groups; 4.9% in the group assigned the antimicrobial soap and 4.2% in the control group. Children showered daily under the supervision of monitors, and were re-examined after one and two months.

At the end of one month, the infection rate was nearly identical in the two groups (6.3 and 6.2%). By the end of two months, the incidence of infection had increased further in both groups, but children in the control group had a slightly lower incidence (8.1%) than those children who had been using the antimicrobial soap (9.0%). Taplin (1981 *Arizona study*) attributed the increase in incidence of infection in both groups over the two-month study period to the "usual summer increase" noted in previous years. He concluded however, that the antimicrobial soap had no effect on the incidence of dermal infection.

According to Taplin (Taplin, 1981 *Arizona study*) dormitory supervisors reported that during the summer of the study, although there was the usual "summer increase" in minor skin infections, the children had "... the lowest incidence of [skin] sores in recent memory." Taplin speculated that this was a consequence of the daily hygiene regimen.

Chlorhexidine Trial Described by Taplin

A collaborative research effort between investigators from the University of Miami and the University of Costa Rica was undertaken to study the effect of a solution of CHX gluconate when applied daily to a population of school children (Taplin, 1981 *Costa Rica study*). All students from two schools in a rural Costa Rican town participated in the study. The average age of the students was nine years. Individuals were assigned to the treatment group or the control group at random. Five days a week for six weeks, each child was sprayed from neck to feet with either a solution of 2% CHX and 4% isopropanol in distilled water (treatment group), or distilled water containing only 4% isopropanol (control group). The volume of solution applied to each child was not specified. Prior to treatment, each child was examined and all skin infections were recorded. Additional examinations were repeated twice a week for the duration of the study. All infections were cultured and the causative microorganisms identified.

At the beginning of the study, the two groups of children had a nearly identical incidence of infected lesions; 10.6% in the group selected for CHX treatment and 10.4% in those selected for the control group. Over the course of the study, 15 children in the CHX group and 38 children in the control group developed newly infected skin lesions. This difference is statistically significant ($p < 0.001$). Significant differences ($p < 0.001$) were also found between the two groups in the total number of newly infected lesions and in the total number of separate infection episodes over the course of the study.

There is no question that Taplin (1981 *Costa Rica study*) clearly demonstrated that CHX was effective in preventing skin infections in a noninstitutionalized population of children. It is not clear, however, if these results can be extrapolated to the population at large (which contains people of all ages and races), or more specifically, to U.S. soldiers in the field. The group of children studied by Taplin and his coworkers were drawn from the same socioeconomic class, were racially similar, and represented a relatively narrow age class. None of these characteristics (i.e., similar race, age, and socioeconomic class) apply to the present U.S. military population, so it is unclear whether the results described by Taplin (1981 *Costa Rica study*) could be replicated in a racially and ethnically diverse adult population.

Antimicrobial Ingredients Available for Use by the U.S. Army

Any antimicrobial soap or similar product selected by the U. S. Army for use in the field should possess a number of characteristics. These include

- Demonstrated safety and efficacy when used routinely on the entire body;
- A high degree of substantivity;
- Activity against gram-positive and gram-negative bacteria, yeasts, and other fungi; and
- The ability to remain effective over a wide range of environmental conditions.

In the following section, we discuss what is known about each of these factors for the four antimicrobial agents available today: CHX, TCC, CFL, and TCS.

Chlorhexidine (CHX)

The FDA granted ICI Americas, Inc., approval to market CHX as an antiseptic in 1978* (Carroll, 1991). This approval was granted after a comprehensive review of safety and efficacy data. Because of its recognized safety and effectiveness in the prevention of skin infection, our comments on CHX are restricted to other issues of potential concern to the U.S. Army.

A substantial amount of effort has been expended in studying the antibacterial activity of CHX on human skin (see for example Smylie *et al.*, 1973; Taplin, 1981 *Costa Rica study*; Lee *et al.*, 1988; Ayliffe *et al.*, 1988; and Bendig, 1990). The majority of these studies concern the utilization of CHX in clinical, surgical, or other specialized circumstances. Other than Taplin (1981 *Costa Rica study*), there have been no publications concerning the prophylactic effect of routine whole-body CHX use on the incidence of skin infections in a normal population. Nonetheless, the data of Taplin (1981 *Costa Rica study*) proved that CHX can prevent bacterial skin infections when applied for five consecutive days per week over a two-month period. During that study there were no reports of adverse effects among the subjects, although two technicians who applied CHX to the study participants developed some irritation of the hands.

Chlorhexidine is a broad-spectrum topical antibiotic with activity against gram-positive and gram-negative bacteria and the yeast *Candida albicans* (Beeuwkes, 1958; Davies *et al.*, 1954; and Aly and Maibach, 1976). There is no evidence to

* The FDA (FDA, 1974) defines an antiseptic as "A safe, non-irritating antimicrobial-containing preparation which prevents overt skin infection."

indicate that CHX is effective against fungi other than yeast, although there are *in vitro* data that show CHX has activity against the human immunodeficiency virus (Montefiori *et al.*, 1990). The absence of data concerning the effect of CHX on fungi is particularly significant because Allen (1989) identified skin infections from the fungi *Trichophyton mentagrophytes*, *Trichophyton rubrum*, and *Epidermophyton floccosum* as being common causes of morbidity among soldiers in Vietnam.

Because CHX exhibits its greatest activity against gram-positive bacteria, concern was expressed by the Panel (FDA, 1974 and 1978) that long-term use of CHX may contribute to the overgrowth of potentially pathogenic gram-negative bacteria that may be unaffected by the compound. Aly and Maibach (1976) examined the effect of daily use of CHX over a six-month period on the bacterial flora of human skin. From the results of their research, Aly and Maibach (1976) concluded that daily use of CHX resulted in "... no specific selection of bacterial species."

Chlorhexidine does not exhibit significant substantivity, although several studies have shown that detergent solutions of 0.7% and 4% CHX retain their antimicrobial activity over a period of several hours following application (Smylie *et al.*, 1973; Lowbury and Lilly, 1973; Ayliffe *et al.*, 1988; and Bendig, 1990).

Aly and Maibach (1979) presented data from another study designed to examine the activity and residual antimicrobial activity of CHX. In this study, a 0.5% solution of CHX was applied to both hands of human volunteers for five consecutive days. Baseline counts of bacteria were taken from both hands of study participants prior to treatment with the CHX solution. The 0.5%-CHX solution was applied to the hands of the subjects once on test days one, two, and five; two additional times on day two; and three times on days three and four. The antibacterial effects of CHX were evaluated by sampling the right hand of test subjects *immediately* after application of CHX, and comparing the bacterial counts obtained at this point with the baseline count for the same hand. To examine whether CHX has residual antimicrobial activity, bacterial counts were taken from the left hand of the same individuals at *hourly intervals* for a period of six hours after treatment on days one, two, and five. Aly and Maibach (1979) observed a consistent, downward trend in immediate post-wash bacterial counts associated with the use of CHX on the right hand. Compared to baseline values, these bacterial counts were 85, 96, and 98% lower on days one, two, and five, respectively. Furthermore, Aly and Maibach (1979) stated that according to their results, no

significant increase in bacterial growth occurred on the left hand for up to 6 h after washing with the CHX solution.

The studies of Smylie *et al.* (1973), Lowbury and Lilly (1973), Ayliffe *et al.* (1988), Bendig (1990), and Aly and Maibach (1979) do not resolve the question of precisely how long CHX remains active against microorganisms following application. We can infer from the data of Taplin (1981 *Costa Rica study*) that a single application of CHX each day, five consecutive days a week for a period of eight weeks is probably adequate for the prevention of bacterial skin infections—at least under the specific conditions of that study. Whether CHX would remain effective if applied at greater intervals remains unknown. No studies have addressed the issue of whether CHX would be effective under conditions of extreme heat and humidity where an individual is likely to sweat profusely. Questions also remain regarding how differences in climate might affect the optimum frequency of application.

Chlorhexidine can be formulated for application in different concentrations, and various investigators have studied the effectiveness of 0.5% (Aly and Maibach, 1979), 0.75% CHX (Lowbury and Lilly, 1973), 2.0%, (Taplin, 1981 *Costa Rica study*), and 4.0% solutions (e.g., Lowbury and Lilly, 1973; Smylie *et al.*, 1973; Ayliffe *et al.*, 1988; and Byrne *et al.*, 1990). Chlorhexidine has also been formulated in detergent solutions (Aly and Maibach, 1976; and Lowbury and Lilly, 1973) and in alcohol (Taplin, 1981 *Costa Rica study*). For hospital uses, a detergent solution of 4.0% CHX appears to be the most effective formulation (Lowbury and Lilly, 1973; and Smylie *et al.*, 1973). No studies have evaluated the optimum concentration of CHX for frequent whole-body application, nor whether the most appropriate solvent is an alcohol or a detergent. However, Byrne *et al.* (1990) observed that a 4% chlorhexidine detergent solution reduced total bacteria colony counts taken from the skin surface of volunteers by a mean level of 94% following whole-body showering and by a mean level of 71% after whole-body bathing. (The greater reduction in the mean bacterial count after showering appears to be due to the removal of bacteria by the mechanical force of the shower stream.) Additionally, Kaiser *et al.* (1988) reported reductions specifically in mean staphylococcal colony counts between 81 and 89% on skin sites of hospital patients 8 to 14 h after a whole-body shower using a chlorhexidine gluconate solution (presumably 4% chlorhexidine); a reduction in mean staphylococcal colony counts between 98% and 99% was also reported on the skin sites of the hospital patients immediately after a second whole-body shower that was taken the morning after the first shower and also involved the use of a similar chlorhexidine gluconate solution.

Finally, as pointed out in our review of Taplin (1981 *Costa Rica study*), it is not clear that CHX would retain its efficacy if applied under less standardized conditions than those described by Taplin (1981 *Costa Rica study*). Furthermore, CHX cannot be formulated as a bar soap (it is cationic and thus is not compatible with soaps, which are anionic) and must be applied as a solution (see Marzulli and Bruch, 1981). This presents problems for the transport and application of CHX in a military combat setting. For example, practical difficulties such as the use of a dispensing system in showering facilities would need to be addressed.

Triclocarban (TCC)

Triclocarban (TCC) was one of several antimicrobial ingredients in the bar soaps evaluated by Leonard (1967), Dubow and Winter (1967), Duncan *et al.* (1969), MacKenzie (1970), and Taplin (1981 *Arizona study*), and was one of a series of carbanilides with antimicrobial activity evaluated for safety and efficacy by the Panel (FDA, 1974 and 1978). Relatively little toxicity or efficacy data on TCC has been published in the open scientific literature, but several pharmaceutical companies submitted data developed in their laboratories to the Panel (FDA, 1974 and 1978). Based on a review of these data, the Panel (FDA, 1978) determined that the information available at that time was not sufficient to reach a conclusion concerning the safety and efficacy of TCC. The FDA Commissioner noted, however, that the evidence presented to him (FDA, 1974 and 1978) did not indicate that the use of TCC presented any known hazard to the general public. However, the Commissioner went on to cite concerns regarding the possibility that TCC and/or its metabolites were associated with testicular lesions in rats. Because TCC can be absorbed through the skin (FDA, 1974 and 1978), the Commissioner concluded in a Tentative Final Order that, pending final determination, the concentration of TCC in bar soaps should not exceed 1.5% (FDA, 1978).

Two months after publication of that Tentative Final Order (FDA, 1978), the FDA received the results of a series of pharmacology and toxicology studies of TCC (Herrmann, 1978, cited in Marzulli and Bruch, 1981). These studies included an evaluation of the toxicity of TCC administered topically or by mouth to infant monkeys; the results of metabolism studies in rats and monkeys; pharmacokinetic studies in rats, monkeys, and humans; and teratology and reproductive studies in rats. Marzulli and Bruch (1981) reviewed these data and concluded that the results demonstrated that a formulation of 1.5% TCC in bar soap was safe for long-term use.

No clinical trial was ever conducted of TCC alone, although as noted above, TCC was one of several antimicrobial ingredients in the soaps used in the studies by Leonard (1967), Dubow and Winter (1967), Duncan *et al.* (1969), MacKenzie (1970), and Taplin (1981 *Arizona study*). Because TCC was always present in a mixture of other antimicrobial ingredients in these clinical trials, no conclusions on the activity of TCC can be drawn from the results of those trials.

There are some published data that indicate TCC has activity against gram-positive bacteria (Roman *et al.*, 1957; Finkey *et al.*, 1984; and Yackovich and Heinze, 1985). For example, Roman *et al.* (1957) reported that TCC had a high degree of activity against a series of gram-positive bacteria *in vitro*. These researchers also demonstrated that the percent reduction in the bacterial flora of hands increased linearly with increasing concentration of TCC over a range of 0.5 to 2.0%. Wilson (1970) confirmed that a bar soap with 2% TCC was an effective bactericide.

Yackovich and Heinze (1985) employed the "agar patch test" technique in two different experimental protocols to evaluate the activity and substantivity, respectively, of a 1.5%-TCC formulated bar soap on human skin. The agar patch test is an *in vivo* procedure that involves streaking bacteria onto the surface of an agar medium. The agar medium is then placed in contact with human skin, which may or may not be treated with an antimicrobial soap. The bacteria on the surface of the agar medium are thereby placed in contact, for a specified amount of time, with any germicidal agent present on the skin. The agar patch is then removed, incubated for 24 h at 35°C, and the colonies that develop on the medium are counted.

In the first experiment, Yackovich and Heinze (1985) attached agar patches streaked with *Staphylococcus epidermidis* to the inside forearm of human subjects. Prior to attachment of the agar patch, the subjects' forearms underwent supervised washings with bar soaps containing either a 1.5%-TCC formulation or a placebo. After eight supervised washings, over a period of two to four days, Yackovich and Heinze (1985) documented a 79 to 88% decrease in the geometric mean number of colony-forming units (CFU)* among those individuals who had used the soap with the 1.5%-TCC formulation (results did not differ for contact times of the agar patch of as little as 30 min or as long as 4 h). This decrease was statistically significant ($p \leq 0.025$).

* Colony-forming units (CFU) is a term typically used to indicate the number of separate bacterial groups or colonies on a solid medium (e.g., agar plate or membrane filter) resulting from replication of a corresponding number of individual organisms.

The main purpose of the second experimental protocol employed by Yackovich and Heinze (1985) was to investigate if an accumulation of TCC on the skin could be attributed to the number of applications of the 1.5%-TCC formulated bar soap. In this experiment, the agar patch test procedure was applied to 39 subjects. These subjects washed with a 1.5%-TCC formulated bar soap between 5 and 13 times over the course of 2 to 4 d. At least two hours were allowed to elapse between each washing. Only after seven or more washings were statistically significant reductions in the number of CFU observed. The magnitude of the effect did not increase beyond that observed after seven washings. Because the magnitude of the reductions was not distinguishable statistically after 7 washes, Yackovich and Heinze (1985) suggested that the amount of TCC deposited on skin reached its maximum after 7 washes and remained there through 13 washes. From the results of this experiment these researchers concluded that repeated washings with a 1.5%-TCC formulated bar soap can lead to the deposition and accumulation of TCC on the skin.

Finkey *et al.* (1984) compared the antimicrobial efficacy of bar soaps formulated with 0.8 or 1.5% TCC. A 0.3-mL solution of the test material was applied to the forearm of each subject and allowed to remain in place for one minute. The area was then rinsed for 30 seconds with tap water. In one set of experiments, the forearms of study participants were washed with acetone prior to application of the soap to remove skin-surface lipids (delipidization). The second series of experiments did not utilize this technique. In both instances, the soap solution was applied seven times over a three-day period. Following the final wash, a 0.1-mL suspension of either *Streptococcus pyogenes*, *Staphylococcus aureus*, or *Corynebacterium minutissimum* (all are gram-positive bacteria) was applied to the skin and the arm was occluded for five hours. Bacteria were removed from the arm by a detergent scrub method, and samples of these bacteria were plated on agar. The number of CFUs was determined following incubation of the plates for 48 h. In the portion of the study that utilized acetone delipidization, the bar soap with 1.5% TCC had statistically significant antimicrobial activity against *Staphylococcus aureus* ($p = 0.00005$) and *Corynebacterium minutissimum* ($p = 0.03$) compared to the placebo-treated site. No conclusions could be drawn regarding *Streptococcus pyogenes* due to the poor survival of this bacterium at both test and control sites. The second series of experiments (similar to the first, except acetone delipidization was not used) provided evidence that both concentrations of TCC exhibited statistically significant ($p \leq 0.05$) activity against *Staphylococcus aureus* and

Corynebacterium minutissimum. There was no significant difference in the magnitude of antimicrobial activity between the two concentrations of TCC.

Data from a series of experiments with radiolabelled TCC indicate that TCC is limited in its penetration of the skin, and has limited substantivity (Black *et al.*, 1975a). For example, human skin biopsies taken ten minutes following a single wash with a solution of 0.08% ¹⁴C-labelled TCC showed "low or very low" amounts of ¹⁴C-labelled TCC in the epidermis or dermis ($\leq 0.090 \mu\text{g}/\text{cm}^2$). Autoradiography of skin biopsies taken after six washes with the ¹⁴C-labelled TCC suspension showed no evidence of accumulation from repeated application, although small amounts of ¹⁴C-labelled TCC were still detectable in the epidermis and dermis 96 hours after the final wash. Black *et al.* (1975a) did not examine whether the quantity of ¹⁴C-labelled TCC that was present 96 hours post-washing was sufficient to retain any antimicrobial activity.

Many questions concerning the activity and substantivity of TCC remain unanswered. We have found no data that indicate TCC is active against gram-negative bacteria, fungi, or other nonbacterial skin pathogens. Thus it is difficult to speculate on TCC's ability to prevent a broad spectrum of microbial infections of the skin. Triclocarban's efficacy in long-term use remains an unresolved issue, and the substantivity of TCC also requires further examination. Finally, there are the questions of how the antimicrobial activity and substantivity of TCC vary, if at all, under the wide range of environmental conditions that could be encountered by U.S. military personnel.

Cloflucarban (CFL)

Cloflucarban was one of a mixture of antimicrobial ingredients in the soaps evaluated by Leonard (1967), Duncan *et al.* (1969), and Taplin (1981 *Arizona study*). Like TCC, no clinical trial has ever been conducted using a soap with CFL as the only active ingredient. Because CFL was always present in a mixture of other antimicrobial ingredients, no conclusions on the activity of CFL alone can be drawn from the results of the clinical trials cited above. In the Tentative Final Order issued regarding O-T-C antimicrobial ingredients (FDA, 1978), the Commissioner of the FDA concluded that CFL is effective as an antimicrobial ingredient when added to soap, but that data were not adequate to make a final determination regarding the safety of CFL for frequent human use. However, enough data were submitted to allow the Commissioner to conclude in 1974 and again in 1978 that there was no

known hazard to the public from the continued use of CFL in antimicrobial soap (FDA, 1974 and 1978).

As explained in the section on active ingredients in antimicrobial soaps, CFL was to be allowed to remain on the market for two years following publication of the Panel's findings as a Final Order. During this time, manufacturers would presumably conduct studies to resolve some of the questions raised by the FDA review. Among the specific gaps in the data base on CFL cited by the FDA were information on substantivity, the extent of absorption of CFL across the skin, peak blood levels of CFL following multiple showers or baths, the rate of metabolic conversion and excretion of CFL from the body, and information on its distribution in various organs and tissues, as well as its potential for tissue accumulation (FDA, 1978). We found no studies on CFL that were published following the FDA reviews (FDA, 1974 and 1978). No antimicrobial-soap bar currently on the market contains CFL, and it is possible that the battery of studies required by the FDA were not pursued by soap manufacturers.

Triclosan (TCS)

Triclosan (TCS) is a substituted diphenyl ether that has been added as an antimicrobial ingredient to bar soaps, skin antiseptics, skin-wound cleansers, and skin-wound protectants (FDA, 1974 and 1978). Like TCC and CFL, the safety and efficacy of TCS were the subject of a comprehensive evaluation by the Panel (FDA, 1974 and 1978). The Panel was concerned that TCS might cause photosensitization of the skin similar to that observed with the halogenated salicylanilides. Although the Panel concluded that TCS is not a primary photosensitizing agent, the Panel found that data were not adequate to determine whether photosensitivity could develop from use of TCS following prior sensitization with other compounds. The Panel (FDA, 1974 and 1978) raised a number of additional questions regarding the safety of TCS on the basis of data that are insufficient or entirely lacking. These questions include

- The potential teratogenicity, carcinogenicity, and reproductive toxicity of TCS;
- Determination of a no-observed-effect level of TCS for all routes of administration;
- The extent of dermal absorption, especially in infants;

- The degree of substantivity; and
- Whether use of TCS will selectively promote the growth of *Pseudomonas aeruginosa* and other gram-negative bacteria.

The Panel (FDA, 1978) concluded that TCS could remain on the O-T-C market in antimicrobial bar soaps for two years following publication of a Final Order. Meanwhile, the Panel recommended that studies be conducted to resolve the questions listed above (FDA, 1978). Because of concerns regarding the extent to which TCS is absorbed across the skin of infants, the Commissioner of the FDA (FDA, 1978) stipulated that antimicrobial soaps that contained TCS must be labelled with a warning to prohibit their use on infants under six months of age. Pending determination of a no-observed-effect level of TCS, the Commissioner also stipulated that antimicrobial soaps must not contain TCS in concentrations greater than 1.0% (FDA, 1978). In contrast to CFL, TCS is still sold for O-T-C use in antimicrobial soaps, and a few published studies (Lilly and Lowbury, 1974; Ayliffe *et al.*, 1988; Bartzokas *et al.*, 1983; and Black *et al.*, 1975b) have examined specific aspects of either its antimicrobial activity or substantivity. However, no publications have described the effectiveness of routine whole-body bathing or showering with a soap containing TCS.

Lilly and Lowbury (1974) evaluated the antibacterial activity of two different formulations of TCS. They found that repeated use (six handwashings, three each on two successive days) of a bar soap with 0.75% TCS resulted in a significantly greater decrease ($p < 0.05$) in the bacteria population on the hands than use of a plain (unmedicated) bar soap. Populations of skin bacteria were not significantly affected by a single handwashing with the TCS soap. The one-time use of a washing cream with 2% TCS was similarly ineffective, while multiple handwashings with the cream again were found to produce significant reductions ($p < 0.001$) in the number of bacteria on the hands. Ayliffe *et al.* (1988) have also shown that a single use of any of three separate products (a bar soap with 1.5% TCS, a washing cream with 2% TCS, and a "skin cleanser" with 2% TCS) were no more effective than plain soap in reducing bacterial populations on the hands of volunteers.

Lilly and Lowbury (1974) also presented data that showed that a cleansing cream with 2% TCS had marked residual activity against *Staphylococcus aureus* (a gram-positive bacteria) and *Escherichia coli* (a gram-negative bacteria) one hour after application. Bartzokas *et al.* (1983) showed that a detergent solution of 2% TCS

retained its antimicrobial activity against *Klebsiella aerogenes* (a gram-negative bacteria) for two hours following three successive handwashes.

Black *et al.* (1975b) evaluated the deposition of ^3H -labelled TCS in human skin following single or multiple (six) washes with solutions of 0.08% ^3H -labelled TCS. The TCS was solubilized in freshly prepared soap, soap prepared one week prior to the experiment, or in a "non-soap" detergent. Biopsies taken immediately after application of TCS showed that relatively little ^3H -labelled TCS remained on the epidermis: 0.058 to 0.110 $\mu\text{g}/\text{cm}^2$ after a single wash, and 0.104 to 0.186 $\mu\text{g}/\text{cm}^2$ after multiple washes. The quantity of ^3H -labelled TCS measured in the dermis was even less and varied from 0.018 to 0.021 $\mu\text{g}/\text{cm}^2$ after a single wash, and from 0.022 to 0.055 $\mu\text{g}/\text{cm}^2$ after repeated washes. Black *et al.* (1975b) considered these values to represent "low to very low" quantities of TCS, but did not interpret them with respect to their potential antimicrobial activity.

The data of Lilly and Lowbury (1974) provide limited evidence that TCS is active against both gram-positive and gram-negative bacteria. However, these studies used only one or two species of each group of bacteria, and cannot be considered comprehensive. There are no data on the activity of TCS against yeast and other fungi, or against viruses. Moreover, the activity of TCS against bacteria has been observed only following multiple applications (Lilly and Lowbury, 1974; Ayliffe *et al.*, 1988; and Bartzokas *et al.*, 1983). While these data indicate that TCS exhibits some substantivity, they also suggest that a threshold concentration is necessary for antimicrobial activity. This apparent threshold concentration has not been characterized. We found no publications on TCS that addressed the other issues raised by the FDA (1974 and 1978), nor are we aware of any data that describe the effectiveness of TCS under different environmental conditions.

Discussion and Research Recommendations

Table A-3 contains a summary of data on the application and effectiveness of CFL, TCS, TCC, and CHX. From a review of this table and the preceding discussion, it is apparent that many questions remain about the prophylactic effect of whole-body use of any of these compounds on the incidence of skin infections preventing related performance degradation in military personnel. As a result, there are a wide variety of research projects that we recommend to the U.S. Army.

In addition to the specific research recommendations listed below for CFL, TCS, TCC, and CHX, the U.S. Army may wish to pursue research designed to compare the effect of showering with water alone (no soap) and showering with

Table A-3. Summary of data on the application and effectiveness of commercially-available antimicrobial ingredients and the antiseptic CHX.

Ingredient	Demonstrated effectiveness in clinical trials	Human toxicity	Active against					Substantivity ^a		
			Gram-positive bacteria	Gram-negative bacteria	Yeast	Other fungi	Viruses	Period of activity ^b	Effect of environment ^c	Optimum concentration
Antimicrobial:										
CFL	ND ^d	ND ^d	Equivocal	Equivocal	ND ^d	ND ^d	ND ^d	ND ^d	ND ^d	ND ^d
TCS	ND ^d	ND ^d	Equivocal	Equivocal	ND ^d	ND ^d	ND ^d	ND ^d	ND ^d	ND ^d
TCC	ND ^d	Equivocal	Yes ^{e,f,g}	ND ^d	ND ^d	ND ^d	ND ^d	ND ^d	ND ^d	Equivocal (range of 0.5 to 2.0%) ^{e,h,j}
Antiseptic:										
CHX	Yes ⁱ	No	Yes ^{k,l,m}	Yes ^{k,l,m}	Yes ^{k,l,m}	ND ^d	Yes ⁿ	ND ^d	ND ^d	Equivocal (range of 0.75 to 4.0%) ^{e,f,i,j,o}

^a Substantivity refers to the persistence of the chemical on the skin after rinsing.

^b Period of activity refers to the length of time after application that the substance retains antimicrobial activity.

^c Role of environment refers to the effect(s) that different environments may have on the substantivity of an antimicrobial substance.

^d ND = not determined.

^e Smylie *et al.* (1973).

^f Ayliffe *et al.* (1988).

^g Roman *et al.* (1957).

^h Finkey *et al.* (1984).

ⁱ Yackovich and Heinz (1985).

^j Taplin (1981 *Costa Rica study*).

^k Beuwkes (1958).

^l Davies *et al.* (1954).

^m Aly and Maibach (1976).

ⁿ Montefiori *et al.* (1990).

^o Lowbury and Lilly (1973).

water and plain soap on populations of skin microorganisms. Data from such studies would provide the U.S. Army with baseline information that could be used to evaluate more accurately the changes in microbial populations that result from the use of antimicrobial soaps or CHX.

Research Recommendations for CFL

When the FDA reviewed the safety and efficacy of CFL, the Commissioner concluded it was an effective antimicrobial ingredient when added to soap (FDA, 1974 and 1978). At the same time the Panel published a formidable list of unanswered questions concerning the pharmacology and potential toxicity of CFL to humans (FDA, 1974 and 1978). To the best of our knowledge, these questions remain unanswered, and to resolve them would require a substantial research effort. Furthermore, even if the toxicological and pharmacological questions were resolved satisfactorily, it would still be necessary to conduct a clinical trial with CFL to ascertain its safety and efficacy in routine whole-body use. In combination, the safety studies and a clinical trial represent a substantial commitment of time and money. However, if the U.S. Army is interested in the use of CFL as an antimicrobial ingredient of bar soap, research should be conducted to resolve the following issues:

- The period of substantivity of CFL under average conditions in a temperate climate, and how substantivity is affected by different climates and microclimates (such as that produced by clothing);
- The optimum concentration of CFL for routine whole-body use;
- The optimum frequency of application of CFL under different environmental conditions;
- CFL's activity (if any) against gram-positive and gram-negative bacteria, yeast and other fungi, and viruses;
- The rate and extent to which CFL is absorbed across the skin;
- The peak blood levels of CFL in adults following single and multiple baths and showers, and how these blood levels compare to those determined by the FDA to be safe (FDA, 1974 and 1978);
- The rate and extent of metabolic conversion and elimination of CFL;

- The distribution of CFL in organs and tissues, and the potential for tissue accumulation; and
- Whether the safety and efficacy of CFL can be demonstrated in clinical trials.

Research Recommendations for TCS

As was the case for CFL, the Panel (FDA, 1974 and 1978) raised a number of questions regarding the safety of TCS, including its potential for teratogenicity, carcinogenicity, and reproductive toxicity. Although TCS remains available in O-T-C products, there are no studies in the open scientific literature that have addressed the specific issues raised by the Panel (FDA, 1974 and 1978). The few studies published since the Panel's review have examined only TCS's substantivity and activity against bacteria (Lilly and Lowbury, 1974; Ayliffe *et al.*, 1988; Black *et al.*, 1975b; and Bartzokas *et al.*, 1983). No clinical trial of TCS has ever been conducted; consequently, there is a broad range of research options that exist regarding TCS. If the U.S. Army is interested in the use of TCS as an active ingredient of antimicrobial bar soaps, the following issues should be examined:

- The potential teratogenicity, carcinogenicity, and reproductive toxicity of TCS;
- Determination of a no-observed-effect level of TCS for all routes of administration;
- The extent of dermal absorption of TCS;
- The period of substantivity of TCS under average conditions in a temperate climate, and how substantivity is affected by different climates and microclimates (such as that produced by clothing);
- The optimum concentration of TCS for routine whole-body use;
- The optimum frequency of application under different environmental conditions;
- TCS's activity against gram-positive and gram-negative bacteria, yeast and other fungi, and viruses, and/or whether use of TCS selectively promotes the growth of *Pseudomonas aeruginosa* and other gram-negative bacteria; and

- Whether the safety and efficacy of TCS can be demonstrated in clinical trials.

Research Recommendations for TCC

Triclocarban has been added to antimicrobial bar soaps as an active ingredient for decades (Leonard, 1967; Dubow and Winter, 1967; Duncan *et al.*, 1969; MacKenzie, 1970; Taplin, 1981 *Arizona study*; and FDA, 1974 and 1978). It remains a common ingredient of bar soaps marketed O-T-C.

The safety concerns about TCC raised by the FDA (1974 and 1978) appear to have been resolved by the data of Herrmann (1978, cited by Marzulli and Bruch, 1981). Nonetheless, there are a number of questions regarding the efficacy of TCC that remain unanswered. The U.S. Army may be interested in conducting research on TCC to address the following issues:

- The extent of TCC's activity against gram-positive and gram-negative bacteria, yeast and other fungi, and viruses;
- The safety and efficacy of TCC in routine, whole-body use;
- The period of substantivity of TCC under average conditions in a temperate climate, and how substantivity is affected by different climates and microclimates (such as that produced by different fabrics);
- The optimum frequency of application of TCC under different environmental conditions; and
- The optimum concentration of TCC for routine whole-body application.

Research Recommendations for CHX

More data are available on the antiseptic CHX than on any of the other antimicrobial substances considered here. The clinical trial conducted in Costa Rica by Taplin and his coworkers (Taplin, 1981 *Costa Rica study*) provided clear evidence of CHX's ability to prevent bacterial skin infections under the specific conditions of the study. Chlorhexidine has the desired attribute of being active against gram-positive and gram-negative bacteria as well as yeast (Beeuwkes, 1958; Davies *et al.*, 1954; and Aly and Maibach, 1976). However, no one has examined whether CHX affects *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Epidermophyton floccosum* or other common disease-causing fungi—which were a significant

problem to combat troops in Vietnam (Allen, 1989). Experiments designed to resolve whether CHX has any ability to inhibit or kill these fungi (as well as viruses) would be of immediate and direct interest to the U.S. Army. For CHX to be used routinely by U.S. Army personnel in a combat zone, the following issues would also require resolution:

- The most appropriate solvent (detergent or alcohol) for CHX for use under field conditions;
- The optimum concentration of CHX for routine whole-body application;
- The most convenient and effective means of applying CHX in the field;
- The period of substantivity of CHX under average conditions in a temperate climate, and how substantivity is affected by different climates and microclimates (such as that produced by clothing); and
- The optimum frequency of application of CHX under different environmental conditions.

Conclusions

Soaps have been used in bathing or showering for decades because they increase the removal of dirt, oil, and microorganisms compared to water alone (Kooymann and Halberstadt, 1945). Antimicrobial ingredients do not enhance the ability of soap to remove dirt or oil, but antimicrobial bar soaps and similar products (like the antiseptic, CHX) can decrease populations of certain microorganisms on the skin. Whether these population decreases are sufficient to prevent microbial diseases of the skin is still an open question for the antimicrobial substances TCC, TCS, and CFL. Only CHX has been shown to have prophylactic activity against bacterial infections of the skin (Taplin, 1981 *Costa Rica study*). It is not known whether CHX is effective against nonbacterial diseases of the skin (other than that caused by the yeast *Candida albicans*), or whether CHX would be effective when used by U.S. military personnel under a variety of environmental conditions.

Although significant questions remain about the efficacy of TCC, TCS, and CFL, TCC may be of potential use to the U.S. Army. The most recent data on this substance indicate that repeated use of a bar soap with either 0.08 or 1.5% TCC caused statistically significant reductions in populations of the gram-positive bacteria *Staphylococcus aureus* and *Corynebacterium minutissimum* (Finkey et al., 1984)

and *Staphylococcus epidermidis* (Yackovich and Heinze, 1985). *Staphylococcus aureus* is an important pathogenic species of bacteria, and was one of the principal causes of bacterial skin infections among U.S. combat troops in Vietnam (Allen, 1989). Whether the reductions in *Staphylococcus aureus* reported by Finkey *et al.* (1984) are medically significant, and whether these reductions can be achieved under conditions in the field remains unknown. Nonetheless, the data of Finkey *et al.* (1984) and Yackovich and Heinze (1985) indicate that TCC does have antibacterial activity. Data from the same two studies also show that TCC has substantivity over a period of ≤ 5 h. Furthermore, TCC has been added to commercially-available antimicrobial bar soaps for years with no apparent adverse effects (FDA, 1978; and Marzulli and Bruch, 1981). Considered together, these data indicate that use of an antimicrobial bar soap with 0.08 or 1.5% TCC poses little if any human health risk, and *may* provide some protection against infections caused by gram-positive bacteria.

Analysis of data on the effectiveness of antimicrobial substances such as TCC and CHX would be facilitated by obtaining information on the effect of showering with water alone (no soap), and showering with water and plain soap, on populations of skin microorganisms. Data of this nature are fundamental to any comprehensive evaluation of changes in microbial populations that may result from the use of antimicrobial soaps or CHX.

Appendix A. References

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Appendix B

Microorganisms That May Induce Skin Diseases

Table B-1 contains a list of those microorganisms identified in the clinical text, *Dermatology, Volume 1, Second Edition* [S. L. Moschella and H. J. Hurley, Eds. (1985), W. B. Saunders Company, Philadelphia, PA] as inducing common infections of the skin. Where the text edited by Moschella and Hurley (1985) cites a primary reference for the relationship between a microorganism appearing in Table B-1 and a specific skin disease, we identify that reference in the table and give its citation in the footnotes.

Table B-1. Microorganisms that may cause skin diseases and therefore may be of significance to the U. S. military.

Type of microorganism	Scientific name	Reference
Bacteria	<i>Acinetobacter calcoaceticus</i>	a
	<i>Actinomyces israelii</i>	b
	<i>Aeromonas hydrophila</i>	b
	<i>Aeromonas aerogenes</i>	b
	<i>Bacterioides corrodens</i>	c
	<i>Bacterioides fragilis</i>	c
	<i>Bacterioides melaninogenicus</i>	c
	<i>Bartonella bacilliformis</i>	b
	<i>Clostridium perfringens</i>	d
	<i>Clostridium welchii</i>	b
	<i>Corynebacterium acnes</i>	e
	<i>Corynebacterium diphtheriae</i>	b
	<i>Corynebacterium minutissimum</i>	f
	<i>Corynebacterium tenuis</i>	g
	<i>Enterobacter cloacea</i>	h
	<i>Enterobacter aerogenes</i>	b
	<i>Erysipelothrix rhusiopathiae</i>	b
	<i>Escherichia coli</i>	b
	<i>Francisella tularensis</i>	b
	<i>Hemophilus spp.</i>	b
	<i>Klebsiella pneumoniae</i> (also identified in literature as <i>Klebsiella aerogenes</i>)	b, c
	<i>Mycobacterium marinum</i>	b
	<i>Mycobacterium ulcerans</i>	b
<i>Pasteurella multocida</i>	b	
<i>Peptococcus spp.</i>	d	
<i>Peptostreptococcus spp.</i>	d	

Table B-1. continued.

Type of microorganism	Scientific name	Reference
Bacteria (continued)	<i>Proteus mirabilis</i>	i
	<i>Pseudomonas aeruginosa</i>	j
	<i>Pseudomonas cepacia</i>	k
	<i>Pseudomonas maltophilia</i>	b
	<i>Pseudomonas pseudomallei</i>	b
	<i>Serratia marcescens</i>	b
	<i>Staphylococcus aureus</i>	l
	<i>Staphylococcus cohnii</i>	b
	<i>Staphylococcus epidermidis</i>	m
	<i>Staphylococcus saprophyticus</i>	b
	<i>Staphylococcus warneri</i>	b
	<i>Streptobacillus moniliformis</i>	b
	<i>Streptococcus bovis</i>	b
	<i>Streptococcus faecalis</i>	b
	<i>Streptococcus pneumoniae</i>	n
	<i>Streptococcus pyogenes</i>	b
	<i>Streptococcus salivarius</i>	b
Fungi	<i>Candida albicans</i>	b
	<i>Candida tropicalis</i>	b
	<i>Phaeoannellomyces wernecki</i> (formerly identified as <i>Cladosporium wernecki</i>)	o
	<i>Cladosporium mansonii</i>	p
	<i>Epidermophyton floccosum</i>	b
	<i>Microsporum audouinii</i>	q
	<i>Microsporum canis</i>	q
	<i>Microsporum distortum</i>	b
	<i>Microsporum ferrugineum</i>	b
	<i>Microsporum gypseum</i>	b
	<i>Pityrosporon orbiculare</i>	r
	<i>Pityrosporon ovale</i>	s

Table B-1. continued.

Type of microorganism	Scientific name	Reference
Fungi (continued)	<i>Trichophyton concentricum</i>	b
	<i>Trichophyton ferrugineum</i>	b
	<i>Trichophyton mentagrophytes</i>	b
	<i>Trichophyton rubrum</i>	b
	<i>Trichophyton schoenleini</i>	b
	<i>Trichophyton tonsurans</i>	b
	<i>Trichophyton verrucosum</i>	b
	<i>Trichophyton violaceum</i>	b
	<i>Trichosporon capitatum</i>	b, t
Viruses	<i>Herpes simplex</i>	u
	<i>Papilloma sp.</i>	b
Other	<i>Leishmania tropica</i>	b

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