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FUNGICIDAL BEHAVIOR OF LAUNDERING DETERGENTS AS A FUNCTION OF WATER TEMPERATURE

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FUNGICIDAL BEHAVIOR OF LAUNDERING DETERGENTS AS A FUNCTION OF WATER TEMPERATURE

by

Georgette Durand

A Dissertation Submitted to the Faculty of the Graduate School at The University of North Carolina at Greensboro in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy

Greensboro 1983

Approved by

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The effects of varied detergent types and concentrations and of three laundering water temperatures on the sanitation of a typical 50 percent polyester/50 percent cotton blend sheeting were studied. Water temperatures selected were $43^\circ$, $51^\circ$, and $60^\circ$C. Six detergent types were chosen on the basis of distinctive components in their formulations; Tide, Oxydol, All, Biological Bold, Wisk, and Dynamo. The three detergent concentrations corresponded to half the recommendation of the manufacturer, the exact recommendation, and twice the recommendation.

Sterile fabric specimens were inoculated with a broth culture of the fungus *Trichophyton mentagrophytes* and laundered at the regular wash cycle in a top-loading heavy-duty home washer. After laundering, fabric swatches were cut and deposited on RODAC plates. Contaminated fabric swatches were counted to determine fungal survival after laundering treatments. Data derived from RODAC counts were statistically analyzed using the analysis of variance procedure.

Results confirmed that water temperature is the most significant variable among those chosen for the study. Fungicidal effect of water temperature is most evident at $60^\circ$C. High survival rate was noticed at $51^\circ$C, except for detergent type Wisk, which was superior to the five other detergents. At $43^\circ$C all fabric swatches were contaminated. Increasing the detergent concentration did not cause the same reaction on all detergent types. When considering the mean values for all
detergent types, more fungal survival was observed at the high detergent concentration which corresponds to twice the recommendation of the manufacturer.

The null hypotheses related to water temperature and to detergent type were both rejected. A statistically significant difference in the variance of fungal survival was found to exist between the interaction of water temperature and detergent type, of detergent type and detergent concentration, and of water temperature, detergent type, and detergent concentration. The null hypotheses concerning detergent concentration and the interaction of water temperature and detergent concentration were supported by the findings of the study. The hypotheses were tested at the 0.05 probability level.
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CHAPTER I
INTRODUCTION

Laundering fabrics was for many years a very simple task. Nowadays, laundering is related to many views and controversies concerning safety, ecology, consumerism, energy, allergies, infection, and others (Norwick, 1976). Changes in laundering practices have to take place since a large proportion of the wash load contains synthetic fibers and has received a permanent press treatment. Due to all these factors consumers have to make various decisions concerning the laundering process to obtain satisfactory results.

Statement of the Problem

The energy shortage and increasingly higher fuel prices have forced attention to energy conservation measures in the home. Since laundering requires energy, specific recommendations have been made to reduce energy usage. For example, suggestions to launder more clothes per load and to launder with low water temperature rather than with hot water have been made. These suggestions unquestionably lead to energy and money savings (Jokelainen & Heino, 1981) but the effect of laundering with lower water temperature on sanitation has not been thoroughly investigated. It should be studied since cold water laundering is common and will probably continue to grow in popularity (Lyng, 1978; Parikh, Connor, Steinke, Brandt, & Avery, 1981).
The survival of potential pathogens on laundered fabrics has become a significant public health concern especially for hospital laundries, for commercial laundries, and for public accommodations such as hotels and motels where the number and pathogenicity of contaminants are of particular concern (Wiksell, Pickett, & Hartman, 1973). With the increased usage of self-service laundry facilities, dissemination of pathogenic microorganisms may be more frequent. Customers using the laundering equipment cannot know who utilized it before or whether the person laundered contaminated fabrics. Even for home laundering, sanitation should be assured, especially when there is illness in the family. In all these circumstances, reduced temperature laundering must be approached with a great deal of caution ("Reduced Temperature Washing," 1978). Energy conservation is justifiable but the sanitary condition of fabrics may as a result be sacrificed for the sake of reducing the amount of energy consumed in heating water (Faig, 1978).

Energy saving is not the only reason why low water temperature is chosen for laundering. The advent of the permanent press fabrics from thermoplastic fibers frequently requires low temperature laundering techniques to maintain their quality and appearance (Nicholes, 1970). Energy saving, fabric treatment, and growth in use of synthetic and of colored fabrics are all reasons why lower water temperature is adopted for laundering.

The increasing usage of cold water in laundering has encouraged investigators to work on microbiological problems related to this practice. In fact, if cold water temperature is utilized by many industries
and consumers, something may have to be done to assure better sanitation of fabrics and, consequently, to increase protection of human beings.

Laundering at low water temperatures requires a new generation of products such as new synthetic detergents that are more adapted to low water temperature laundering. For some time, there have been several detergents on the market that promise good performance at water temperature in the range of \(45^\circ\) to \(50^\circ\) C, but consumers launder with even colder water temperatures especially during winter months in the northern parts of North America. Whether or not the detergent manufacturers will succeed in further lowering the temperature requirements for efficiency remains to be seen. Consumers should be informed on which type of detergent and on how much detergent they should use with water temperature colder than what they formerly chose for laundering in order to achieve optimum removal of pathogenic microorganisms.

In summary, sanitation through laundering is an actual issue. It is imperative to favor research in an area that is so directly related to human health.

**Objectives**

The objectives of this study are the following:

1. To determine the effect of water temperature on survival of the fungus *Trichophyton mentagrophytes*, a commonly encountered fungal pathogen.

2. To evaluate the importance of detergent type on survival of the fungus *Trichophyton mentagrophytes* on fabric laundered in low water temperature.
3. To investigate the role of detergent concentration on survival of the fungus *Trichophyton mentagrophytes* on fabric laundered in low water temperature.

4. To examine any interaction between type and concentration of detergent, and different water temperatures on the survival of the fungus *Trichophyton mentagrophytes* on fabric after laundering.

**Hypotheses**

The following hypotheses will be tested:

1. There will be no significant difference in fungal contamination after laundering at different water temperatures.

2. There will be no significant difference in fungal contamination after laundering with different types of detergent formulations such as nonionic versus anionic surfactants; presence of enzymes versus no enzymes; presence of a builder versus no builder; and presence of oxygen bleach versus no oxygen bleach.

3. There will be no significant difference in fungal contamination after laundering with different detergents over a range of concentrations.

4. There will be no significant interaction between detergent types, detergent concentrations, and water temperatures on the survival of the fungus on the laundered fabric samples.
Assumptions

The following assumptions are made:

1. Fabric selected is representative of fabrics most often laundered on a weekly basis.
2. Fabric dye does not affect fungus growth.
3. Detergents chosen in each category are representative of other detergents of the categories under investigation and are also representative of detergents more commonly utilized by consumers.
4. Water temperatures selected are representative of water temperatures available in different areas of North America.
5. Organism chosen is representative of the pathogens commonly encountered in laundering and will produce generalizable conclusions.

Definition of Terms

Amphoteric Surfactant

A synthetic surface active agent that may be anionic, cationic, or that may have no net charge depending on the pH of the aqueous solution ("The SDA Consumer," 1981).

Anionic Surfactant

A synthetic surface active agent in which detergent properties depend in part on the negatively charged anion of the molecule. Its activity is greater at alkaline pH. Linear alkylate sulfonate is the most popular anionic surfactant.
**Bleach**

An ingredient that cleans, whitens, brightens, and removes stains from fabrics when added to laundry detergents. Powdered oxygen bleach, such as sodium perborate, is often utilized since it can be used safely on most fabrics, colors, and fabric finishes unlike chlorine bleach ("The SDA Consumer," 1981).

**Builder**

A material that enhances the cleaning action of the surfactant by sequestration, by precipitation, or by ion exchange. Builders supply alkalinity, act as buffers, prevent redeposition of soil during laundering (Cowan, 1980; "Laundry Soaps," 1976).

**Cationic Surfactant**

A synthetic surface active agent in which detergent properties depend in part on the charged cation of the molecule. Its activity is greater at acid pH. Quaternary ammonium compounds, such as alkyl dimethyl benzyl ammonium chloride, are the most commonly used cationic surfactants.

**Enzymes**

Large and complex molecules which are members of the protein family. They act as proteolytic catalysts on protein stains. Since they need more than the regular wash period of fifteen minutes to be effective, they are more often utilized in presoaking products.
**Fungicide**

An agent capable of killing fungi when conditions are favorable.

**Fungus**

A microorganism which has no chlorophyll and which reproduces by means of spores. Molds, mildews, and mushrooms are classified as fungi (Alexopoulos & Mims, 1979).

**Laundering**

A method of removing soil from fabrics and of controlling transmission of microorganisms by fabrics.

**Nonionic Surfactant**

A synthetic surface active agent that contains neither positively nor negatively charged functional groups. It does not ionize in solution. Its activity is not affected by acids, alkalis, or by hard water. Ethoxylated alcohols and alkyl amine oxides are commonly used. Nonionic surfactants are very effective in removing oily soil ("The SDA Consumer," 1981).

**Sanitation**

A measure directed toward providing and maintaining an environment in which it is safe for animals and for human beings to live.

**Synthetic Detergent**

A surface active agent made from petroleum, fatty acids, and other sources. It retains its cleaning power even in hard water. A synthetic detergent may be built or unbuilt and may also contain
ingredients such as antiredeposition agents, suds control agents, corrosion inhibitors. The finished product can be in a number of forms, such as granules, liquids, and crystals ("The SDA Consumer," 1981).
CHAPTER II
REVIEW OF RELATED LITERATURE

A considerable number of studies have been reported over the years concerning the spread of microorganisms on fabrics following the laundry process. This review of related literature presents, first, the conditions for fungus growth. Secondly, the contamination of fabrics by fungi is discussed. Finally, the author considers the different laundering and drying conditions for fungi control, such as laundering equipment, laundering and drying methods, water temperature, detergent type and concentration, and other factors pertaining to fabric care.

**Conditions for Fungus Growth**

Different factors influence the growth of fungi. They need food, suitable pH, and temperature. The optimal conditions are not the same for all types of fungi. Most fungi grow between 0° and 35° C, but the optimum temperature range is 20° to 30° C (Alexopoulos & Mims, 1979). Organisms, such as *Trichophyton mentagrophytes*, grow at temperatures between 11° and 35° C with an optimum temperature between 20° to 28° C (Wilson, Mizer, & Morello, 1979).

Some fungi adapt more easily and survive even in unfavorable environmental conditions. In contrast to bacteria, fungi prefer an acid medium in which to grow. A pH of six is about the optimum needed...
Light is not essential for the growth of fungi although it is required, in many species, for sporulation.

Fungi have different food requirements. Some are omnivorous and can subsist on almost anything that contains organic matter. Others, such as dermatophytes, obtain their food by infecting living organisms as parasites. Lacking chlorophyl, fungi require already elaborated food in order to live. Given carbohydrates in some form, preferably glucose or maltose, most fungi utilize inorganic or organic sources of nitrogen to synthesize their own proteins (Alexopoulos & Mims, 1979).

It is important to be aware that textile materials can be carriers for saprophytic and pathogenic microorganisms. In fact, many kinds of diseases may be transmitted by clothing. Any type of textile material should be treated in such a way that, following laundering and drying, it becomes free of pathogen microorganisms.

Contamination of Fabrics by Fungi

According to Ross (1979), different factors contribute to the rise in interest in the growth of parasitic fungi on human beings. This is due to the fact that medical practitioners can now identify symptoms caused by the growth of fungi rather than attribute some skin disease to bacterial infection as it was often diagnosed in the past. Also, the rapid rise in human population resulting in increased density in living conditions tends to favor the spread of fungal infections to a greater degree than in less populated areas. In fact, when human population is more dense, adequate sanitation is oftentimes more difficult (Ross, 1979). The increased usage of public laundering facilities is another
factor which may cause an increase in contamination of fabrics by fungi.

Fungi Causing Skin Infection

Fungi resemble simple plants. They reproduce by means of spores. Usually, they are filamentous and multicellular. The filaments constituting the body of a fungus elongate by apical growth, but most parts of an organism are potentially capable of growth, and a minute fragment from almost any part of the fungus is able to produce a new growing point and to start a new individual. The branching filaments or hyphae grow on the surface or within a host, either spreading between the cells or penetrating into them. However, the hyphae cannot grow significantly into the deeper or living layers of a host; they spread radially into adjacent areas of skin (Alexopoulos & Mims, 1979; Frobisher & Fuerst, 1973).

Fungi attacking human beings are usually classified into three groups based on the extent of the infection they cause. These are the superficial or cutaneous, subcutaneous, and systemic mycotic diseases (Wilson, Mizer, & Morello, 1979). Superficial mycotic diseases or dermatomycoses do not invade the deeper tissues or internal organs of man. Fungi live on the horny layer of the skin and exude an enzyme. This enables them to digest keratin causing different reactions on the body (Domonkos, 1971; Morton, 1965; Sauer, 1973).

Dermatomycoses are the most common type of fungus diseases in man. They are caused by members of a group of fungi called dermatophytes. Other names are also given to identify dermatomycoses
depending on the location of the infection on the body (Frobisher &
Fuerst, 1973; Ross, 1979; Sauer, 1973). Superficial fungus infections
are usually chronic, are difficult to treat, and are directly trans­
missible by close or indirect contacts between infected individuals
(Wilson et al., 1979).

There are three important genera of dermatophytes: Microsporum,
Epidermophyton, and Trichophyton. Wilson et al. (1979) reported that a
particular species of dermatophytes may cause a variety of clinical
lesions in different areas of the body, and different fungi may cause
similar clinical symptoms. Pathogenic fungi are often present in hairy
parts of the body, under nails, and in skin folds. The soles of the feet
rarely support fungi but many species of fungi invade the skin between
toes.

Superficial fungus infections of the skin, hair, and nails are
known by the medical term tinea. Specific names are given to tinea
depending on where the infection occurs on the body (Domonkos, 1971;
Frobisher & Fuerst, 1973). For example, when fungus infection occurs on
the scalp, it is called tinea capitis; when it is on the body, the
specific name is tinea corporis. Tinea barbae identifies a fungus
infecting the beard, and tinea unguium or onychomycosis is the fungus
disease attacking the nails. When fungus infection appears on the
feet, a condition commonly called "athlete's foot," the infection is
designated as tinea pedis (Alexopoulos & Mims, 1979; Domonkos, 1971;

Three species of fungi belonging to two different genera, Trichophyton
and Epidermophyton, can cause tinea pedis. These are Trichophyton
mentagrophytes, Trichophyton rubrum, and Epidermophyton floccosum. Trichophyton mentagrophytes causes the majority of the cases of tinea pedis, according to Domonkos (1971). It produces an acutely inflammatory condition and the vesicular eruption tends to spread. With the formation of vesicles, burning and itching occur until they open and release a fluid. Oftentimes fissures between the toes become secondarily infected with pyogenic cocci which cause great discomfort and may be incapacitating (Domonkos, 1971; Morton, 1965). Trichophyton rubrum causes a relatively noninflammatory type of tinea. The eruption occurs near a fungus-infected toenail or between or under the toes, on a hand or other parts. This species, mentioned by English (1969) and Sauer (1973), shows a great resistance to treatment.

When Epidermophyton floccosum invades the feet, it spreads and produces scaling and some vesiculation. The disease on the feet, says Domonkos (1971), is more often restricted to the toes although the entire sole may become infected. Ross (1979) reported that these fungi are responsible for some of the most widespread and cosmopolitan diseases of human beings. Due to the fact that in most cases there are no serious complications, they are not regarded as important diseases. The person having "athlete's foot" usually seeks relief only when acute lesions develop (Domonkos, 1971). Some dermatologists believe that almost everyone carries the causal fungi of dermatomycoses on symptom-free feet and that the lesions appear only when there is a change in the person's resistance which favors fungi proliferation. The majority of dermatologists support the theory that a few people are carriers of
the causal fungi and that the majority of people are either free of the microorganisms or else have lesions of the skin, of the nails or both (English, 1969).

Sources of Contamination

Skin fragments, nails, and hair from an infected person are important sources of contamination, stated English (1979) and Ridenour (1950). Skin infection such as tinea pedis and other tinea can be transmitted from one person to another especially when people walk barefooted in public places like swimming pools. English (1969) reports that tinea pedis is a problem of countries with high living standards due to the fact that a person picks up a fungal inoculum while walking on surfaces contaminated with skin fragments and then incubates the fungus by wearing socks and shoes for hours, providing in that way ideal conditions for fungal growth--dark and moist atmosphere in which secretions accumulate ("How to Stamp," 1977; Waller & Mercer, 1980; Wilson et al., 1979).

Socks are not the only articles causing contamination. Towels, sheets, and other items of wearing apparel are also possible sources of disease transmission. When clothing is close-fitting, nonabsorbent, or infrequently laundered, similar results occur. Even if the transmission of human fungus infection caused by Trichophyton mentagrophytes is not yet completely understood, some authors state that clothing is a means of transference of that fungus. Clinical observations have verified the assumption that pathogenic fungi are transmitted through fabric (McNeil & Greenstein, 1961).
Wearing improperly laundered items of apparel which were contaminated with *Trichophyton mentagrophytes*, or with any other fungi, is dangerous since cross-contamination may occur (Warden & Highley, 1974). Thus, it is important to take different precautions to avoid disease transmission. For example, it is advisable not to shake dirty clothes near clean laundry or near surfaces on which laundry is sorted after laundering. Different authors mention the danger of cross-contamination within a load itself (Ridenour, 1950; Stritzke, 1971). Since contamination can also occur between succeeding batches of clothes in the same washer, Ridenour (1950) suggested the use of a disinfectant between batches. This same advice is given to families who utilize public facilities to launder.

It is also recommended that known infected garments be laundered separately. Many studies have shown that when heavily contaminated materials were laundered in the same load with noncontaminated ones, the amount of organisms present on the material approached an equilibrium due to redeposition. The length of the wash cycle, water temperature, and detergent concentration influence the end result (Ridenour, 1950; Stritzke, 1971; Warden & Highley, 1974). Contamination produced by the same variables was observed in the case of both the fungus *Trichophyton mentagrophytes* and the bacterium *Staphylococcus aureus*, the latter being considered tenacious (Witt & Warden, 1971).

**Laundering and Drying Conditions for Fungi Control**

Research done by different authors such as Arnold (1938), Khan and Roch (1981), McNeil and Greenstein (1961), Ridenour (1950), and
many others emphasize the importance of laundering to control disease transmission. Several variables such as laundering equipment, laundering and drying methods, presence of soiling, fabric type, water temperature, detergent type and concentration, and addition of disinfectant were all observed in order to better understand the relationship between laundering and drying conditions and fungi control.

Laundering Equipment

Important changes have been made in the designing of home laundry appliances since 1972. The newer models are more efficient, reported Schrage (1980), for energy conservation. The author added, however, that this is too often at the cost of reduced performance.

The approach taken by the industry to reduce energy is the use of less hot water. For example, valves for warm water temperature settings have been adjusted from 60 percent hot/40 percent cold water, to 50 percent hot/50 percent cold water, or to 40 percent hot/60 percent cold water. Another measure chosen by many manufacturers is to nearly eliminate the warm rinse option.

Laundering and Drying Methods

Kundsin, Walter, Ipsen, and Brubaker (1963) reported on families utilizing community self-service laundries rather than their own equipment. They found more cases of staphylococcal disease.

When laundering at reduced water temperature, it is important to load the washer properly. If it is overloaded the clothes have to be laundered for a longer period of time to obtain enough mechanical action to clean satisfactorily and, thus, remove microorganisms (Siu,
1980). Many authors mentioned the importance of agitation or mechanical action during laundering for removal of soil and of microorganisms especially when using a short time cycle and low temperature formula (Guernsey, 1926; Kohler, 1954; Meyers, 1968; Spillard, 1964). Another author reported that the number of microorganisms was greater in the rinse than in the wash water which reveals that more agitation during laundering is needed to get rid of microorganisms and to obtain sanitary clothes (McNeil & Choper, 1962b). Stritzke (1971) indicated that an agitation time of more than ten minutes is needed to decrease survival and redeposition of Trichophyton mentagrophytes beyond the amount found after two minutes of agitation.

Results of different investigations show that the best place to kill microorganisms is in the washwheel. Marmo (1969; 1970b) stated that soil and microbial removal occur simultaneously and in the same manner. Microbes affixed to fibers of the fabric and to soil are dislodged from the fabric by mechanical and chemical action and are suspended in the wash solution and rinsed away in the same manner as soil particles.

The wash cycle design has an effect on the number of microorganisms recovered on fabric after laundering (McNeil, 1964; Sidwell, Dixon, Westbrook, Forziati, i971; Wiksell, Pickett, & Harman, 1973). Data indicate, for example, that there was a significant difference among three different wash cycles when water temperature was 49°C (Walter & Schillinger, 1975). When permanent press and regular wash cycles were compared, Wiksell et al. (1973) stated that the greatest reduction in residual numbers of different microorganisms occurred with
the regular wash cycle, which is longer and more vigorous. Although
the laundering process removes a great amount of microorganisms it does
not necessarily kill all of these. Greene (1972) and Marmo (1969;
1970b) reported that unless a fabric is properly cleaned there is some
doubt about its sanitary condition. Specific conditions such as high
water temperature or the use of a disinfectant might be necessary

Various authors commented on the influence of drying on sanita-
tion. Some of them stated that when higher drying temperature was used
more microorganisms were eliminated (Janecek, Manikowske, & Bromel,
1980). That occurred especially when cold water temperature was chosen
for laundering (Janecek et al., 1980; Khan & Riggs, 1980). Khan and Riggs
(1980) also report that the importance of drying temperature is greater
with cold water laundering, and that it is also influenced by the type
of detergent. LaunJering at 21°C with a nonbuilt detergent followed by
air drying produced the highest bacterial count. The majority of inves-
tigators, however, reported that drying temperature has no effect, and
that drying cannot be relied on for sanitation. Organisms remaining in
the dryer can be redeposited on other items dried later (Lyle, 1977;
Marmo, 1968a; Ridenour, 1950; U.S. Department of Agriculture, 1971;
Witt & Warden, 1971). Some authors added that drying is not a substitute
for hot water and for detergent action (Stritzke, 1971; Warden &
Highley, 1974). Stritzke (1971) explained that drying, especially after
washing at low water temperature, does not destroy Trichophyton
mentagrophytes. For example, if clothes are damp when placed in the
dryer, they do not take on the temperature of the dryer, but when the
clothes are hot enough to destroy the microorganisms, the dryer turns off since the clothes are dry.

Some authors considered calendering in their investigations. Results show that if the fabric contains enough moisture when entering the calender, it is an effective method for sanitation especially when it is set at high temperature (Perdue, 1970; Ridenour, 1950; Wagg, 1973).

Presence of Soiling

Some authors mentioned the influence of adding soil on the fabrics while testing for removal of microorganisms. Apparently in contradiction with Marmo (1969), Stritzke (1971) and Warden and Highley (1974) reported that whether or not the fabric swatches were inoculated with soil did not prove to be significantly different. There was no effect on the initial growth of the fungus, on its survival after laundry, or on its redeposition on uninoculated fabrics. The authors did not discover any significant difference in the acceptance of *Trichophyton mentagrophytes* caused by redeposition during laundry whether the uninoculated swatches were soiled or not. Warden and Highley (1974) added that these findings were the same for two types of soil they investigated.

Fabric Type

Another variable called fabric type was also studied by different authors. McNeil and Greenstein (1961) reported that the physical characteristics of the fibers and the electric charge of both fiber and microorganism may influence their attachment. Fabric construction and moisture content are factors influencing the number of microorganisms.
found in and on textiles and clothing. Actual testing shows that the number of organisms removed from different fabrics was statistically different. For example, more microorganisms were removed from a 60% nylon/40% cotton rib knit fabric compared to removal from a 50% wool/30% nylon/20% cotton terry knit fabric. There was also a significant difference between the two fabrics in the degree of survival and redeposition of microorganisms. The differences were noticed at the end of the wash cycle and drying period. The greater amount was found on the wool blend (Witt & Warden, 1971).

Different results, however, were reported by other researchers. For example, Meyers (1968) affirmed that there was no measurable difference, after detailed comparisons, in the performance of all-cotton versus polyester/cotton sheeting.

Other sources stated that no difference could be detected while testing fabrics of various construction types or of different fiber composition ("Hot Water Wash," 1971; Khan & Riggs, 1980; Sidwell, Dixon, Westbrook, & Forziati, 1971). Since contradicting results were obtained from different authors, more research is necessary to clarify the influence of fabric type on microorganism removal and redeposition.

Water Temperature

Considerable information has been reported in consumers' magazines and in specialized journals concerning the necessity of reducing energy usage by lowering water temperature of laundering. As a consequence, different investigations were conducted to clarify the effect of reducing water temperature on detergent efficiency and sanitation.
The preservation of some fabrics, such as silks and woolens, and of some colored fabrics, necessitates the use of low water temperature for laundering (Arnold, 1938). Fabric structure or applied finish are other variables influencing the choice of water temperature for laundering. The temperature of rinse water has to be selected carefully in order to cool down the fabric progressively (Gibbons, 1972). Due to the limits imposed by certain fibers and dyes, Wagg (1973) insists on the fact that colored articles should not be purchased for use in hospitals. These fabrics cannot withstand the high water temperature necessary for disinfection.

Some fabrics need hot washings to become sanitary and that is particularly true for fabrics used in public facilities, such as hotels and motels. The same attention should be given at home when there is sickness in the family or when washing diapers, sheets and other clothing soiled with human waste. In these cases a water temperature of 60°C is recommended ("Hot News About," 1974).

The terms hot, warm, and cold water temperatures do not have the same meaning for everybody, for every place in the world, and for every season of the year. In fact, there is no scientifically accepted point at which water changes from being cold to warm or from being warm to hot. Even water from the tap which is considered cold by most people varies as much as 15°C to 20°C from place to place ("Reduced Temperature Washing," 1978). For example, cold water can vary, reported Lyle (1977), from the freezing point to body temperature. Most water heaters are designed to deliver water temperature in the range of 60°C to 66°C but the actual temperature of hot water in the washing machine is more often...
between 49° and 55°C ("Cold Water Detergent," 1969; Cree1, 1976; McNeil & Greenstein, 1961) and warm water setting is approximately 37°C (U.S. Department of Agriculture, 1971).

Since a wide variety in water temperature exists, it is important to examine its influence on fabric sanitation. Couse (1981) stated that most clothes can be safely washed in cold water without any problems concerning microorganisms. A consumers' magazine reported that the Public Health Service's Center for Disease Control says that the risk of spreading disease in the family is minor. For example, the risk of having "athlete's foot" when socks laundered in cold water are shared by children in the family is small ("Hot News About," 1974). In fact, everybody should be willing to switch to cold water laundering since it saves money and energy. The point is that people need to know if fabrics are as sanitary as when they are laundered in hot water ("Cold Water Detergent," 1969).

Other reports, however, indicated that there may be some problems when laundering in cold water. Potentially infectious and harmful microorganisms are not killed by cold water laundering ("Laundry Detergents," 1978; Mueller, 1978; Pickett, 1973). Arnold (1938) reported that high temperature laundering process destroyed all microorganisms on white clothes. When lower temperatures were used in the laundering of colored clothes harmful microorganisms were not eliminated. Other authors, such as Khan and Riggs (1980) and Manikowske (1977) reported that hot water kills microorganisms or prevents reproduction. Warm and hot water temperatures were compared by many investigators for their capability of reducing the amount of
microorganisms present on fabrics. McNeil (1963), Meyers (1968), Ridenour (1950), and many other researchers reported that laundering and rinsing with hot water resulted in higher removal of microorganisms. The same conclusions were reached by Jaska and Fredell (1980), Walter and Schillinger (1975), and Witt and Warden (1971). Stritzke (1971) was very specific and reported that water temperature was the most important variable in fungal removal, redeposition, and survival, and that the critical water temperature required for the prevention of growth of *Trichophyton mentagrophytes* after laundry was found to be between 49°C and 60°C, and that additional studies are needed to narrow this range of water temperature. According to Stritzke (1971), water temperature has also an effect on the presence of organisms in the washer and the dryer, after their respective cycles. No presence of the fungus was detected either in the wash or rinse water or the washer or dryer at the 60°C or the 49°C water temperature. Fungus was present when a water temperature of 38°C was chosen. Janecek, Manikowske, and Bromel (1980), Sidwell, Dixon, Westbrook, and Forziati (1971), and Warden and Highley (1974) arrived at the same conclusion and added that a warm wash temperature is not high enough in itself to reduce the count of microorganisms to an acceptable level.

Most authors agreed that significant differences were found between the use of cold, warm, and hot water temperatures for laundering and that more microorganisms were removed at the hot water setting. Even when using hot water temperature for laundering, sanitation is not guaranteed especially for the most virulent microorganisms (Guernsey, 1926; McNeil & Choper, 1962b; Witt & Warden, 1971).
Laundering time also influences fabric sanitation. A reduction in time is possible when using hot water temperature. In fact, even a slight increase in temperature can markedly reduce time for killing pathogenic organisms ("Laundry Bacteria Control," 1969; Meyers, 1968). It is very important to take into account the findings which deal with sanitation, especially in the laundering of fabrics used in hospitals, in public facilities, or when utilizing public laundering equipments.

In some countries such as the United States, specific recommendations are given to persons responsible for laundering operations. More specifically, the United States Department of Agriculture recommends that a water temperature as high as 60°C be utilized for home laundering when heavily soiled articles are to be washed, and 49°C for lightly soiled fabrics. For hospitals, washing at 71°C is recommended (U.S. Department of Agriculture, 1979). The Canadian Council of Hospital Accreditation has no statement regarding the temperature at which linen must be washed. As long as the laundry produces linen which is free of contamination and passes the scrutiny of the Infection Control Committee, the requirements of acceptability are met (Murray, 1980).

Water temperature is an important factor for sanitation, especially nowadays due to the publicity about energy saving by using cold water for laundering fabrics. It is evident that cold water laundering saves energy and the decision for using it or not is in the hands of the consumers and institutional laundering industries. It is essential that information be given to consumers on the potential risks taken when using cold water for laundering.
Another factor to consider besides water temperature is the type of detergent utilized for laundering. Detergents are not all the same. Different organic surfactants and builders included in detergents yield varying soil removal characteristics. Some are better for different types of soils, or in various water hardnesses (Bloom, 1980). In fact, a good home laundry detergent is capable of removing many kinds of soils on different types of fibers (Stritzke, 1971). For many years, investigators compared detergents on their ability to clean fabrics. Some authors have looked at the capability of different detergents to get rid of microorganisms.

Because of so many different types of detergents on the market, consumers find it difficult to decide which detergent would best meet their needs. Unfortunately, the decision most often depends on advertisements. Diversification and changes in types of detergents are likely to continue since the availability of raw materials and the need to conserve energy are very likely to lead to new or modified detergent formulations ("Standard Detergent 124," 1981). Nowadays there are granular and liquid detergents. Liquid detergents are built or unbuilt. In the latter case, more surfactant enters in the detergent formulation ("Cold-Water Laundering," 1974). The amount of suds, for granular detergents, is controlled by manufacturers. Some detergents are marketed especially for use with cold water temperature but may be used for warm or hot laundering water temperature.

Another way to classify detergents is by the chemical composition of the basic ingredient, called the surfactant. Oftentimes a detergent
contains more than one kind of surfactant to insure better results. There are anionic, nonionic, cationic, and amphoteric surfactants. Anionic surfactants are most commonly used (Cowan, 1980). Several investigators compared results obtained from different types of surfactants. For example, McNeil and Choper (1962b) and Wilkoff, Dixon, Westbrook, and Happich (1971) reported that the reduction of microorganisms was not affected by using anionic or nonionic detergents. Other sources stated that nonionic detergents are not useful as disinfectants. They have no fungicidal or bactericidal activity because they do not form ions which can disrupt bacterial cell membranes (Janecek et al., 1980; Morin, 1972; Witt & Warden, 1971).

The second main component of synthetic detergents, the builder, was also investigated for sanitizing properties by various authors especially after the phosphate builders had been banned in certain areas in the early 1970's. After that, the level of phosphate has been reduced essentially in all laundry products from 12% to between 3 and 8.5% (Carfagno, 1978; "Cold-Water Laundering," 1974; Purchase, 1972). Different measures were taken to compensate for that modification in detergent formulation. For example, some manufacturers use more sodium citrate or silicate in liquid detergents. Investigators claim that phosphate is a unique builder and that its use reduces the level of microorganisms in fabric thus reducing the risk of infection from fabrics (Jaska & Fredell, 1980; Khan & Riggs, 1980; Stritzke, 1971).

A promising substitute for phosphate is sodium nitrilotriacetate (NTA). In Canada, NTA enters in the formulation of many detergents.
Because of the questions raised concerning its use in large quantities, NTA in detergent was discontinued in the United States for a period of time. Now, NTA usage is allowed by the Environmental Protection Agency, but its future use in household laundry products is hard to predict since consumer groups and congressmen among others feel that it has yet to be proven that NTA is safe enough ("NTA Usage," 1980).

Silicate-built liquid laundry formulations are considered as a reasonable alternate to phosphate-built detergents (Campbell, 1976). Hammond (1971) added that sodium silicate as well as sodium carbonate contribute to deposit of calcium on clothes and on washer parts especially in areas where water hardness is high. Aluminosilicate, a crystalline compound called Zeolite A, is utilized even if it is not, strictly speaking, a builder since it remains insoluble in water throughout the laundering process. It has one of the functions of a builder in softening water through ion exchange. Aluminosilicate, however, does not scavenge magnesium ions (Carfagno, 1978; Cowan, 1980).

Other important ingredients such as bleaching agents enter into the formulation of detergents. A significant change in the proportion of bleaching agents, such as sodium perborate is now noticed. The percentage formerly used was about 10% but is increased to 20 and even 30% in some cases (Hill, 1970; "Washing Powders," 1974).

Enzymes are other possible components of detergents that are compatible with almost all of the other detergent ingredients. They are now used in presoaking and in some detergent formulations. Protease enzymes, which break down proteins, and amylase enzymes for starch are the two enzymes most often added to detergent formulation (Dornbusch,
1978; Purchase, 1972; Soap & Detergent Association, 1982). In the United States few laundry detergents such as Era Plus, Dynamo Action Plus, Axion, and Fresh Start contain enzymes (see Appendix A). Presently, no enzyme laundry detergent is available in Canada (Echlin, 1981).

Christensen, Holm, and Sønder (1978) compared washing results obtained with and without enzymes. They found that prolonged soaking periods were necessary to obtain adequate results when using cold water washing. Purchase (1972) and Stritzke (1971) reported that it requires at least 30 minutes for enzymes to reach their full effectiveness. According to Dornbusch (1978) and Nielsen, Jepsen, and Outtrup (1981), the effectiveness of enzymes increases with temperature of water up to 71°C. Nielsen et al. (1981) added that where washing temperatures of 10°C to 25°C are predominant, the addition of enzymes has only a marginal effect. In these cases enzymes are only used in special formulations in which a synergistic effect between the enzyme and the special detergent ingredients improve the efficiency.

The variable, type of detergent, is not considered significant by most authors. Ledoux (1978) found that no microorganisms were killed when 21 different detergent trademarks were compared. Jaska and Fredell (1980), Wiksell et al. (1973), and Witt and Warden (1971) stated that although detergent might aid in the physical removal of microorganisms, the variable, detergent type, is not a significant one in reduction of these organisms. Very few investigations were reported specifically on the effect of detergent type on dermatophytes such as Trichophyton mentagrophytes. Thus, more studies are needed to broaden knowledge of the effect of detergent type on fungi.
Detergent Concentration

Janeczek et al. (1980) and Ridenour (1950) stated that on the basis of removal of microorganisms, detergent concentration had no significant effect. In contradiction, other investigators, such as Witt and Warden (1971), reported that as detergent concentration increased, survival and redeposition of microorganisms decreased on fabrics after laundering and drying. When a water temperature of 16°C was utilized, several authors found that detergent concentration was especially important (Morin, 1972; Witt & Warden, 1971).

Detergent concentration also affects significantly the amount of microorganisms recovered from the washer and the dryer after removal of the fabric (Witt & Warden, 1971). After studying the fungus *Trichophyton mentagrophytes*, Stritzke (1971) and Warden and Highley (1974) reported that detergent concentration was a significant factor in fungal survival and redeposition.

Addition of Disinfectant

Several authors claimed that complete disinfection can not be achieved through detergency, removal of soil, or by biocidal effect, or by dilution alone. For example, a textile bacteriologist stated that any disinfectant is better than none when laundering. The data obtained in that study reveal that water temperature influences the efficiency of the disinfectant on reduction of microorganisms ("Laundry Digest," 1966). For example, Buford, Pickett, and Hartman (1977) stated that chlorine and phenolic disinfectants are less effective at 24°C than at higher temperatures such as 51°C. Two other studies report that in cold
water perborate "all-fabric" bleaching agents diminish in effectiveness and that chlorine bleach is still efficient in cold water ("Laundry Digest," 1966; "When and How," 1975). In contradictory studies a zero count of microorganisms was found when a disinfectant was added in nearly all instances regardless of water temperature (Janecek et al., 1980; "What About Bacteria," 1966).

Janecek et al. (1980) stated that cold water laundering, such as 18°C, necessitates the addition of a disinfectant to augment the killing of microorganisms during laundering. Buford et al. (1977) and McNeil and Choper (1962b) indicated that using sanitizing agents lowers the amount of microorganisms but, more often than not, does not destroy all the organisms.

Marmo (1970a) affirmed that, with high water temperature laundering, a disinfectant is not needed. Heat, according to Wagg (1973), is more efficient than chemicals for disinfection. The same author reported that the efficiency of the chemical is affected by the textiles themselves and by the soiling matter. Chemical concentration and the length of time of the treatment also influence the result. Bleach usage should not be increased in order to compensate for lower temperature ("Controlling Energy," 1978) because of potential damage to coloration.

A problem arises when heat or chemicals can not be used. This is the case for colored fabrics and for synthetic fiber articles. In situations where serious contamination occurs it has been suggested that heat or chemicals be used even if the life of the fabric would be reduced. Safety of the textiles in use is critical in cases of illness (Walter & Schillinger, 1975; Whittall, 1976).
Interactions Among Laundering Conditions

When reporting significant interactions between different variables, investigators related detergent type and water temperature. Different authors reported that when using a phosphate detergent for washing fabrics, the higher the water temperature, the more efficient the detergent. For example, with cold water laundering the phosphate detergent is not very efficient (Jaska & Fredell, 1980; Khan & Riggs, 1980).

Witt and Warden (1971), after studying the interaction between detergent concentration and water temperature, reported that increasing detergent concentration causes a decrease in survival of microorganisms, especially with a water temperature of 49°C compared to 27°C. Morin (1972) reported that when using a water temperature of 60°C for laundering, destruction of microorganisms is nearly complete whether a detergent was added or not. The same author stated that as the temperature increases, the influence of detergent concentration becomes less important.

Morin (1972) commented on the interaction between detergent type and concentration. The author reported a significant interaction between these two variables, mainly at 27°C compared to 49°C or 60°C. For example, an anionic detergent in a concentration of 0.15 percent and of 0.30 percent did significantly reduce the number of microorganisms. When a nonionic detergent at 0.075, 0.15, and 0.30% concentration is used, the lowest degree of survival occurred with the 0.30% concentration. However, the study of Janeček et al. (1980) did not report any significant interaction between detergent type and detergent concentration.
Interaction between water temperature and disinfectant concentration is also considered. Janecek et al. (1980) mentioned that a concentration of 200 parts per million of both a chlorine and of a quaternary ammonium compound results in less count of microorganisms when water temperature was 41°C compared to a water temperature of 18°C.

Summary of Review of Related Literature

Research studies indicate that water temperature is the most important factor in the destruction of microorganisms in the laundering process. When cold water is adopted for laundering, disinfection must rely on other variables aside from water temperature to eliminate contamination due to pathogens such as the fungus Trichophyton mentagrophytes. Detergent type and concentration are among the variables that might influence sanitation especially when laundering with cold water temperature. No study has clearly indicated the possible efficacy on sanitation of the available commercial laundry detergents when used at low washing temperatures.
The fungus *Trichophyton mentagrophytes* was selected as the test organism since the literature substantiates that it is the most common cause of dermatophytosis and a good measure of sanitation. The fungus was used to determine the effects and interactions of water temperature, of commercially available detergent types, and of detergent concentration on the survival of the fungus under study.

The procedures adopted for this study reproduce the home laundry process. In that way, the influence of the variables mentioned above will be measured with realism.

**Inoculating Procedure**

**Test Organism and Maintenance**

The fungus *Trichophyton mentagrophytes* (No. E9129) was obtained from the American Type Culture Collection, Rockville, Maryland. The freeze-dried specimen was recovered following the procedure established by the manufacturer. Secondary cultures were prepared from the main strain by transferring the test culture to agar slants. These were the stock cultures used to inoculate new slants. The original culture was stored in a freezer at \(-4^\circ C\) until needed.

**Fabric Preparation and Sampling**

The fabric selected for this study comprises 50% polyester/50% cotton, representative of fabrics commonly used for sheeting.
It is made by Dominion Textile Company and has a Truprest II™ finish which is a permanent press treatment. The fabric was purchased from H. Maillet, a retailer in Saint-Antoine, Kent, New Brunswick, Canada. The fabric was yellow and weighed 150 grams per square meter and the thread count was 29 warp and 22 filling (per centimeter).

The fabric was divided in seven different groups, one for the launderings without detergent and one for each type of detergent utilized for the study. Then, each group of fabric was prelaundered without detergent or with the type of detergent to which each group of fabric was randomly assigned for the experimental laundering procedure. The amount of detergent recommended by the manufacturer was used for each of the six detergents. This was to insure removal of any temporary finishes and/or soiling which could have been left from manufacturing. Each group of fabric was laundered in water at 52°C along with a wash load weighing two kg. The normal cycle was selected for laundering and the fabric was dried in a automatic dryer for thirty minutes. This conditioning procedure was repeated three times.

Each group of fabric was then boiled for 45 minutes, and cut into swatches of 400 cm². These were selected randomly and coded to indicate the type of detergent that was used for conditioning and to designate subsequent treatments—-the detergent concentration and water temperature. Each fabric swatch was then sterilized.

**Sterilization Techniques**

Distilled water, mycobiotic agar prepared for agar slants and for RODAC plates, nutrient broth for inocula, and solution of yeast
extract for moistening fabric during incubation were all sterilized in a portable Electric Steroclave (autoclave manufactured by Wisconsin Aluminum Foundry Company, Manitowoc, Wisconsin) at 121°C, 15 pounds steam pressure, for 15 minutes.

Most pieces of equipment were sterilized for 30 minutes in the same manner. These were glass jars with stainless steel blades and screw cap covers utilized to homogenize the nutrient broth, one ml. pipettes, glass stirring rods, tweezers, scissors, cotton applicators, and hypodermic syringes and needles. The fabric test samples, previously conditioned, were placed in Mason™ jars, and labeled before being sterilized for 30 minutes in the autoclave following the procedure described above.

Corningware™ incubating jars covered with aluminum foil, were sterilized in a laboratory oven (Despatch Oven Company, Minneapolis, Minnesota) at 165°C for two hours, as suggested by Frobisher and Fuerst (1973). The same sterilization technique was applied for hanger rods that were wrapped in aluminum foil. The rods support the clips holding fabric test samples, in the incubating jar. Finally, clips holding fabric test samples were soaked in anhydrous 95 percent ethanol for ten minutes since they do not tolerate high heat. This procedure was suggested by Frobisher and Fuerst (1973).

**Media**

The fungus *Trichophyton mentagrophytes* was maintained alive in agar slants. The mycobiotic agar (Difco Laboratories, Detroit, Michigan) was selected since it is a medium recommended for the isolation of pathogenic fungi. The composition of this medium is as
follows: (ingredients per liter)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto-Soytone</td>
<td>10.00 g</td>
</tr>
<tr>
<td>Bacto-Dextrose</td>
<td>10.00 g</td>
</tr>
<tr>
<td>Bacto-Agar</td>
<td>15.00 g</td>
</tr>
<tr>
<td>Actidione</td>
<td>0.50 g</td>
</tr>
<tr>
<td>Chloromycetin</td>
<td>0.05 g</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>

The pH, at 3.5% concentration and 25°C, is 6.5.

The mycobiotic agar was rehydrated by suspending the appropriate number of grams in distilled water. The solution was thoroughly mixed in a beaker and heated on a Tek-Stir Hot Plate and Heat-Stir 36 (Tek-Pro Company, Evanston, Illinois). The agar solution was then dispensed into test tubes, covered with absorbent cotton plug, autoclaved, placed at a 15° angle for cooling, and refrigerated at 5°C until needed.

The Sabouraud dextrose broth (Difco Laboratories, Detroit, Michigan) was chosen since it is recommended for the cultivation of yeast, molds, and aciduric microorganisms such as fungi. The composition of this broth is as follows: (ingredients per liter)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neopeptone, (Difco)</td>
<td>10 g</td>
</tr>
<tr>
<td>Bacto-Dextrose</td>
<td>20 g</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

The pH, at 3.0% concentration and 25°C, is 5.7.

Two antibiotics—chloramphenicol and acidione, cycloheximide (United States Biochemical Corporation, Cleveland, Ohio)—were added to the nutrient broth to suppress bacterial growth. The concentration of chloramphenicol was 0.05 mg per ml of medium. The antibiotic was added to the nutrient broth powder. The media were rehydrated in distilled water and heated in the same way as the solution of mycobiotic agar. The nutrient broth, containing the antibiotic, was poured, 90 ml
per 250 ml Erlenmeyer flask, and covered with a foam plug before autoclaving. The concentration of the antibiotic actidione was 0.5 mg per ml of medium. The powder was dissolved in warm distilled water, dispensed into test tubes, ten ml in each, covered with absorbent cotton plug, autoclaved, and stored in a refrigerator at 5°C. The solution was added to the sterilized nutrient broth just before inoculation of the fungus.

**Inoculation**

After seven days of incubation in test tubes, the fungus was transferred to the nutrient broth using an inoculating loop. During incubation the flasks of broth were placed in a Hetotherm shaking water bath (Danemark) running at 85 rpm. Pilot tests determined that a 3% concentration nutrient broth, the water bath maintained at 26°C, and an incubation period of nine days were the best combination of variables for optimum growth of the fungus *Trichophyton mentagrophytes* in this study.

The broth in which developed fungal pellets was transferred to a sterilized jar of a Waring Commercial Blender (Model 7011). A uniform hyphal suspension was obtained after two minutes of agitation. The blender jar was then allowed to stand until the foam had settled. When needed, one ml of the culture was transferred into 100 ml of the new nutrient broth. Then, sterilized fabric swatches were soaked in the hyphal suspension for ten minutes. The excess liquid was then squeezed out by hand. Sterilized surgeons' gloves were utilized during that procedure.
Inoculated fabric swatches were incubated during eight days at room temperature (20° to 22°C) in a sterile Corningware™ jar covered with an aluminum foil. Sterilized foam plugs were inserted in the cover to permit air circulation. The jar was placed in a fume hood to avoid contamination of the room. After three days of incubation the fungus was visible on the swatches. A relative humidity of approximately 70% was maintained in the incubating jar due to the presence of sterilized distilled water in the bottom of the jar. During preliminary studies a hygrometer was suspended inside the incubating jar containing distilled water and fabric swatches soaked in distilled water. Also, a solution of 0.3% concentration of Bacto-Yeast Extract (Difco Laboratories, Detroit, Michigan) in distilled water was applied daily in the form of a fine stream using a sterilized hypodermic syringe and needle. That was to add humidity and B vitamins which promote fungal growth on the swatches. The pH of the solution is 6.8 at 0.3% concentration.

On the first day of the incubation, a small sample of the inoculated fabric was cut with sterilized scissors and placed in a disposable sterile RODAC plate (60 x 15 mm) (Falcon, Oxnard, California) containing mycobiotic agar. Other samples were taken at three-day intervals and at the end of the incubation period to confirm fungal growth.

**Laundering Procedure**

**Laundry Equipment and Cycles**

An automatic top-loading heavy duty home washer of the type recommended by AATCC (test method 124-1978) was utilized for this study.
(Kenmore, Model 4051880). The wash cycle ran for ten minutes. The water was then drained and a high spin speed was followed by four power spray rinses. The washer was then filled for a two-minute agitated deep rinse at approximately 37°C. After water was drained, another spin plus four power spray rinses took place before the final spin which removed excess water. The complete wash cycle lasted for 38 minutes. Approximately 43 liters of water were needed to fill the washer tub for the low water machine setting. A representative of the City Hall in Moncton, where this work was accomplished, reported that the water had a hardness of 11 ppm and a pH of 6.5.

Water Temperature

The desired wash water temperatures were obtained by varying the amount of warm and cold water coming into the washer. Water temperature was taken by immersing a mercury thermometer in the washer. Three water temperatures, 43°, 51°, and 60°C, were chosen for this study.

Detergent Type and Concentration

Six detergents were selected among those available in the supermarkets. They are representative of various combinations of surfactants, builders, and other ingredients such as enzymes and oxygen bleach. The particular products were of the heavy-duty type and two out of the six were liquid detergents. A list of the most important ingredients is presented in Table 1 and in Appendix A. These detergents were compared in terms of their ability to eliminate the fungus *Trichophyton mentagrophytes*. 
Table 1
Detergent Composition

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Company</th>
<th>Surfactant(s)</th>
<th>Builder(s)</th>
<th>Enzyme</th>
<th>Oxygen Bleach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tide</td>
<td>Procter &amp; Gamble Inc.</td>
<td>Anionic</td>
<td>NTA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxydol</td>
<td>Procter &amp; Gamble Inc.</td>
<td>Anionic</td>
<td>NTA</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologica l Bold</td>
<td>Procter &amp; Gamble Inc.</td>
<td>Anionic</td>
<td>NTA</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>Lever Detergents Ltd.</td>
<td>Nonionic</td>
<td>NTA</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phosphate Carbonate Silicate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wisk (USA)</td>
<td>Lever Detergents Ltd.</td>
<td>Anionic</td>
<td>Citrate</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nonionic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dynamo</td>
<td>Colgate-Palmolive</td>
<td>Anionic</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nonionic</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Nitrilotriacetate

Three different detergent concentrations were selected—high, medium, and low. Control washings, without any detergent, were run at each water temperature. The medium concentration corresponds to the manufacturer's recommendation for normal soil, the highest concentration is twice the recommendation, and the low concentration is half the recommendation. Table 2 presents the quantity of detergent needed, the concentration as well as the pH. In all the experiments the pH of the wash water was measured with a Canlab Portable Digital pH Meter (Model H5503-1). A Sartorius precision balance (Model 2842) was utilized to
weigh the four powdered detergents. Liquid detergents were measured with a graduate cylinder.

Table 2
Detergent Quantity, Concentration, and pH

<table>
<thead>
<tr>
<th>Detergent Type</th>
<th>Quantity in 43 liters</th>
<th>Concentration (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tide</td>
<td>39.96 g&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.093</td>
<td>9.65</td>
</tr>
<tr>
<td></td>
<td>72.92 g</td>
<td>0.186</td>
<td>9.85</td>
</tr>
<tr>
<td></td>
<td>159.84 g</td>
<td>0.372</td>
<td>9.95</td>
</tr>
<tr>
<td>Oxydol</td>
<td>37.74 g&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.088</td>
<td>9.55</td>
</tr>
<tr>
<td></td>
<td>75.48 g&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.176</td>
<td>9.70</td>
</tr>
<tr>
<td></td>
<td>150.96 g</td>
<td>0.352</td>
<td>9.90</td>
</tr>
<tr>
<td>Biological</td>
<td>34.34 g&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.080</td>
<td>9.35</td>
</tr>
<tr>
<td>Bold</td>
<td>68.67 g&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.160</td>
<td>9.65</td>
</tr>
<tr>
<td></td>
<td>137.34 g</td>
<td>0.320</td>
<td>9.75</td>
</tr>
<tr>
<td>All</td>
<td>45.67 g&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.106</td>
<td>10.25</td>
</tr>
<tr>
<td></td>
<td>91.35 g&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.212</td>
<td>10.55</td>
</tr>
<tr>
<td></td>
<td>182.71 g</td>
<td>0.424</td>
<td>10.65</td>
</tr>
<tr>
<td>Wisk (USA)</td>
<td>39.38 ml&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.092</td>
<td>8.85</td>
</tr>
<tr>
<td></td>
<td>78.75 ml&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.183</td>
<td>9.30</td>
</tr>
<tr>
<td></td>
<td>157.50 ml</td>
<td>0.366</td>
<td>9.60</td>
</tr>
<tr>
<td>Dynamo</td>
<td>18.96 ml&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.044</td>
<td>7.15</td>
</tr>
<tr>
<td></td>
<td>37.92 ml&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.088</td>
<td>7.25</td>
</tr>
<tr>
<td></td>
<td>75.84 ml</td>
<td>0.176</td>
<td>7.50</td>
</tr>
<tr>
<td>(none)</td>
<td>-</td>
<td>0.000</td>
<td>7.20</td>
</tr>
</tbody>
</table>

<sup>a</sup>Recommended concentration

Experimental Laundry Sequence

Before washing, the inoculated swatches were removed aseptically from the incubating jar and placed on sterile aluminum foil. A sample
of the inoculated fabric was plated to confirm growth of the organism on the day of laundering. Using sterilized tweezers and scissors the swatches were cut into five samples. The dimension of each sample was approximately 80 cm².

The inoculated fabrics were added to a normal wash load consisting of three sheets cut into quarters and weighing approximately two kg. A water volume of 43 liters resulted in fabric weight-water volume ratio of 0.05 kg/l. When the water reached the desired temperature the required amount of detergent was added in the washer containing the sheets; then, the five inoculated swatches were added to the wash load.

Immediately after completion of the wash cycle a one cm² sample of each of the five swatches was aseptically cut and placed in culture plates. Then, the inside surfaces of the washer were swabbed with a cotton applicator for confirmation of fungicidal activity.

Prior to the use, and in between each wash load, the washer was disinfected using 300 ml of chlorine bleach. For this operation the washer was filled with hot water (50°C), at the normal wash cycle, at the high water level and rinsed at approximately 37°C. Periodically, a sterile cotton applicator was used to swab the washer to confirm sterilization.

Combination of Variables

Each set of five inoculated fabrics was laundered separately, as determined by the experimental design. The entire procedure was repeated three times for every combination of the three independent variables. Table 3. illustrates all the laundering conditions for one
type of detergent and each of these conditions was repeated using each type of detergent under study.

Table 3
Combination of Variables for Each Detergent Type

<table>
<thead>
<tr>
<th>Water Temperature (°C)</th>
<th>Detergent Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>43</td>
<td>half the recommendation</td>
</tr>
<tr>
<td>51</td>
<td>half the recommendation</td>
</tr>
<tr>
<td>60</td>
<td>half the recommendation</td>
</tr>
<tr>
<td>43</td>
<td>the exact recommendation</td>
</tr>
<tr>
<td>51</td>
<td>the exact recommendation</td>
</tr>
<tr>
<td>60</td>
<td>the exact recommendation</td>
</tr>
<tr>
<td>43</td>
<td>twice the recommendation</td>
</tr>
<tr>
<td>51</td>
<td>twice the recommendation</td>
</tr>
<tr>
<td>60</td>
<td>twice the recommendation</td>
</tr>
</tbody>
</table>

A total of 162 launderings were needed to study the three variables, 27 launderings for each type of detergent. The three launderings, without any detergent, were run three times, at the three different water temperatures. These nine washings were done to verify whether microorganisms were removed simply by agitation and flushing action during washing.

Plating Procedure

After each laundering sequence, each fabric sample was cut aseptically and one cm² specimens were deposited in RODAC plates containing sufficient mycobiotic agar to cover the bottom of the
petri-dish. Each plate was labeled, inverted and incubated at room temperature for nine days.

The number of RODAC plates in which growth of the fungus *Trichophyton mentagrophytes* could be detected was recorded. The presence of growth of the fungus on the plates obtained from the five inoculated swatches of each laundering treatment was used to determine the survival of the microorganism on fabrics laundered at three water temperatures with different detergent types and over a range of concentrations. After examination, the plates were destroyed by autoclaving.

**Statistical Procedure**

The number of RODAC plates, in which the fungus *Trichophyton mentagrophytes* was detected, was determined for samples washed at three water temperatures, with different detergent types and over a range of concentrations. The data obtained were analyzed using a factorial analysis of variance procedure. A 3 x 6 x 3 factorial design was needed. This statistical analysis was performed on a computer with the SPSS software package. It determined the simple main effects of variables as well as the interactions resulting from the combined influence of the variables. An assessment of the magnitude of the effects was also performed. The effects of the treatments were considered significant at a probability level of 5%.
CHAPTER IV
DATA AND ANALYSIS

The variables studied in this research, as stated in Chapter I, were water temperature, detergent type, and detergent concentration. Three water temperatures, 60°C, 51°C, and 43°C, were chosen along with six types of detergent formulations at various concentrations. The low concentration corresponds to half the recommendation of the manufacturer, the medium concentration is the exact recommendation and, finally, twice the recommendation of the manufacturer is the high concentration. Some launderings without detergent were also performed to check if dilution alone is capable of destroying the fungus *Trichophyton mentagrophytes*. No treatment was considered to have had a significant effect on fungal survival unless the probability level for that treatment was under five percent.

This chapter will be divided into seven sections: effect of water temperature; effect of detergent type; effect of detergent concentration; interactions of water temperature and detergent type; detergent type and detergent concentration; water temperature and detergent concentration; and finally, water temperature, detergent type and detergent concentration.

Appendix B tabulates actual numbers of fabric swatches on which the fungus *Trichophyton mentagrophytes* survived after each laundering treatment. As can be seen, each treatment was repeated three times. Averages of the number of contaminated fabric swatches laundered at
three water temperatures, with six detergent types, and at three detergent concentrations are shown in Table 4.

<table>
<thead>
<tr>
<th>Detergent Type</th>
<th>Mean Number of Contaminated Fabrics</th>
<th>Percentage of Contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tide</td>
<td>3.04</td>
<td>60.8</td>
</tr>
<tr>
<td>Oxydol</td>
<td>3.19</td>
<td>63.8</td>
</tr>
<tr>
<td>All</td>
<td>3.56</td>
<td>71.2</td>
</tr>
<tr>
<td>Biological Bold</td>
<td>3.52</td>
<td>70.4</td>
</tr>
<tr>
<td>Wisk (USA)</td>
<td>2.52</td>
<td>50.4</td>
</tr>
<tr>
<td>Dynamo</td>
<td>3.37</td>
<td>67.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Detergent Concentration</th>
<th>Mean Number of Contaminated Fabrics</th>
<th>Percentage of Contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>3.13</td>
<td>62.6</td>
</tr>
<tr>
<td>Medium</td>
<td>3.20</td>
<td>64.0</td>
</tr>
<tr>
<td>High</td>
<td>3.26</td>
<td>65.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Water Temperature</th>
<th>Mean Number of Contaminated Fabrics</th>
<th>Percentage of Contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>60°C</td>
<td>0.63</td>
<td>12.6</td>
</tr>
<tr>
<td>51°C</td>
<td>3.96</td>
<td>79.2</td>
</tr>
<tr>
<td>43°C</td>
<td>5.00</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Overall Mean After Laundering with Detergent: 3.20 64.0
Overall Mean After Laundering Without Detergent: 3.67 73.4

a Maximum number is 5.00 or 100% contamination
b 27 launderings for each detergent type
c 54 launderings for each detergent concentration and for each water temperature
Effect of Water Temperature

A statistical analysis of the data showed that water temperature is a very important factor in fungal survival. Figure 1 illustrates clearly that as water temperature increased, survival of *Trichophyton mentagrophytes* decreased. The significance of the fungicidal effect of water temperature, as reported in Table 5, was highly superior to the probability level of 0.05 accepted for this study. This is evidenced by an F value of 536.79 for the main effect water temperature. Thus, the null hypothesis concerning water temperature has to be rejected.

At 60°C, 12.6% of fabric swatches were contaminated after laundering which is less contamination than with the two other water temperatures (see Table 4). The significant effect of hot water temperature laundering upon the survival of microorganisms agrees with results reported by McNeil and Choper (1962a), Sidwell et al. (1971), Stritzke (1971), and many others. Laundering without bleaching at or above 60°C provides satisfactory removal of bacteria for health care facilities reported Jaska and Fredell (1980) and Walter and Schillinger (1975). The data gathered in the present experiment support these previous studies, and it is obvious that water temperature below 60°C may compromise freedom from bacteriological and fungicidal qualities.

Even if laundering with a water temperature of 51°C reduces significantly fungal survival compared to results obtained for laundering at 43°C, it does not produce a high level of decontamination. In the present study the mean percentage of contaminated swatches was 79.2% after laundering at 51°C, and at 43°C all fabric swatches
Figure 1. Mean number of contaminated swatches after laundering at three water temperatures.
Table 5
Three Way ANOVA for Variable Detergent Type, Detergent Concentration, and Water Temperature

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main Effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detergent type</td>
<td>20.198</td>
<td>5</td>
<td>4.040</td>
<td>7.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Detergent concentration</td>
<td>0.457</td>
<td>2</td>
<td>0.228</td>
<td>0.43</td>
<td>0.648</td>
</tr>
<tr>
<td>Water temperature</td>
<td>563.160</td>
<td>2</td>
<td>281.580</td>
<td>536.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>2-Way Interactions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detergent type by detergent concentration</td>
<td>22.136</td>
<td>10</td>
<td>2.214</td>
<td>4.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Detergent type by water temperature</td>
<td>33.877</td>
<td>10</td>
<td>3.388</td>
<td>6.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Detergent concentration by water tempera-</td>
<td>1.395</td>
<td>4</td>
<td>0.349</td>
<td>0.66</td>
<td>0.618</td>
</tr>
<tr>
<td>ture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>3-Way Interactions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detergent type by detergent concentra-</td>
<td>45.790</td>
<td>20</td>
<td>2.290</td>
<td>4.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tion by water temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With detergent vs. without detergent</td>
<td>1.877</td>
<td>1</td>
<td>1.877</td>
<td>0.41</td>
<td>0.524</td>
</tr>
</tbody>
</table>
were heavily contaminated (see Table 4). From this experiment, as well as for the study reported by Wiksell et al. (1973), it is obvious that laundering at 51°C or at 43°C could be hazardous for home laundering especially if there is sickness in the family.

Effect of Detergent Type

The six detergent types utilized in this experiment differed in their ability to remove the fungus Trichophyton mentagrophytes. The percentage of fabric swatches contaminated after laundering varied between 50.4 to 71.2% (see Table 4). Figure 2 shows clearly the superiority of the liquid detergent Wisk (USA) whose builder is sodium citrate and which contains both an anionic and a nonionic surfactant (see Table 1). The enhanced fungicidal effectiveness of that detergent for the fungus Trichophyton mentagrophytes can probably be attributed to its formulation.

Tide, whose surfactant is anionic, is the second best detergent in its capability to remove the fungus, when compared to other detergents (see Table 4). The only detergent in the study having only a nonionic surfactant and various builders is the detergent All. It is the detergent whose pH is the highest, between 10.25 and 10.65 depending on the concentration. As can be seen in Figure 2 and Table 4, it is among the three poorer detergents in terms of fungal removal. This result is in conformity with previous studies conducted by Janecek et al. (1980) and Morin (1972), who found that laundering at low water temperature with a nonionic detergent was not as good as with an anionic detergent when considering decontamination.
Figure 2. Mean number of contaminated swatches after laundering with six different detergent types.
The analysis of variance reveals that the variable detergent type has a highly significant effect on the number of contaminated fabric swatches surviving laundering. This may be observed from Table 5. The F value for detergent type is 7.70 which leads to a significance less than 0.001 and to rejection of the null hypothesis concerning the variable detergent type.

To further analyze the effect of the variable detergent type, the mean number of contaminated fabric swatches after laundering with all of the six detergents was compared to the one obtained from fabric laundered without any detergent (see Table 4). The lower mean number for contaminated fabrics laundered with detergents may be the result of a lower surface tension causing physical removal of the fungus *Trichophyton mentagrophytes*. However, the difference in fungal survival between these two means was not significant at the 0.05 level due to a high variance within detergent types (see Table 5). If one considers the results for individual test temperatures, there may be an effect of detergent at the intermediate temperature, 51°C.

**Effect of Detergent Concentration**

The mean numbers of contaminated fabric swatches after laundering with low, medium, and high detergent concentration vary from 62.6 to 65.2 percent (see Table 4). The low detergent concentration produced the greatest reduction in fungal survival among the three detergent concentrations which is not necessarily the case for all detergent types.
The mean survival of *Trichophyton mentagrophytes* at different detergent concentrations was not found to be significant by the F test for variance. The F value of 0.43 with two degrees of freedom lead to a significance of only 0.648 (see Table 5). Thus, the null hypothesis that contamination was the same at all concentrations could not be rejected at the 0.05 level. Janecek et al. (1980), Manikowske (1977), and Ridenour (1950) arrived at the same conclusion when they studied the effect of detergent concentration on fabric decontamination.

**Interaction of Water Temperature and Detergent Type**

Figure 3 shows the relation between the two variables—water temperature and detergent type. All combinations of detergent type and water temperature at 60°C reduced considerably the mean number of contaminated fabric swatches compared to the results obtained after laundering at 51°C or 43°C. Laundering at 60°C with Tide, an anionic detergent, produced the highest degree of fabric decontamination with a mean number of contaminated swatches of 0.11.

The difference between detergent types appears to result mainly from the increased effectiveness of the Wisk detergent which is composed of both anionic and nonionic surfactants and of sodium citrate as a builder. For that detergent, laundering at a water temperature of 51°C resulted in a much higher degree of decontamination at medium and high concentrations compared to all other detergent types analyzed in this study. Water temperature of 51°C appears to have some effect on the fungicidal activity for two detergent types, Tide at medium and high
Figure 3. Mean number of contaminated swatches as related to detergent type and water temperature.
concentrations and Oxydol at low concentration. With All, Dynamo, and Biological Bold there is not much change from the results obtained at 43°C where decontamination was not observed for all detergent types (see Figure 3). Although results obtained for all detergent types prove that a water temperature of 60°C is needed to insure a high degree of decontamination, laundering at 60°C is not often feasible. In fact, the hot water setting of the home washer is usually between 52°C and 57°C which does not assure fungal decontamination. Thus, sanitation is not guaranteed even when using the best detergent type.

A statistically significant difference in the variance of fungal survival was found to exist between the interaction of water temperature and detergent type. In previous studies Jaska and Fredell (1980) and Khan and Riggs (1980) obtained the same result. From Table 5 it may be seen that the F value of 6.46 with a degree of freedom of ten leads to a significance less than 0.001 between these two variables. This shows that differences in fungal survival among the three laundering water temperatures employed vary over the chosen detergent types in a way that chance alone cannot easily explain. Thus, the null hypothesis concerning the interaction between water temperature and detergent type has to be rejected.

**Interaction of Detergent Type and Detergent Concentration**

The effect of detergent concentration on fungal survival varies with detergent type as illustrated in Figure 4. For example, with the two best detergent types of the study, Wisk and Tide, the medium
Figure 4. Mean number of contaminated swatches as related to detergent type and detergent concentration.
detergent concentration presented the most satisfactory destruction of the fungus *Trichophyton mentagrophytes*. The medium detergent concentration corresponds to the exact recommendation of the manufacturer.

The low detergent concentration, or half the manufacturer's recommendation, gave better results for the four other detergents—Oxydol, All, Biological Bold, and Dynamo. In the case of the detergent Oxydol, an anionic detergent containing oxygen bleach, there is an important difference between the degree of decontamination obtained with the low detergent concentration compared to the one with the two other detergent concentrations.

None of the six detergent types showed better decontamination with the high detergent concentration. It seems that the high detergent concentration, which is twice the recommendation of the manufacturer, is less effective in decontaminating fabric swatches. A plausible explanation is that when there is more detergent in the washer tub less mechanical action is possible; this has been proved to be true for soil removal (Davis, 1963).

The analysis of variance demonstrates a significant interaction of detergent type and detergent concentration. Table 5 indicates that an F value of 4.22 with a degree of freedom of ten leads to a significance less than 0.001 and, consequently, to the rejection of the null hypothesis. Results prove that the effect of detergent concentration varies significantly with detergent types.
Interaction of Water Temperature and Detergent Concentration

It is evident from the data that, whatever the detergent concentration, fungal destruction is always better when fabric is laundered at a water temperature of 60°C compared to the results obtained at the two other water temperatures (see Appendix B). Detergent concentration seems to have little effect on fabric decontamination at water temperatures of 51°C and 43°C. The only apparent difference between the three detergent concentrations occurs when the fabric swatches are laundered at a water temperature of 60°C. This result is in contradiction with findings reported by Stritzke (1971). That author pointed out that detergent concentration is of greater significance at lower water temperatures such as 38°C and 49°C compared to 60°C.

Detergent concentration did not result in a significant interaction with water temperature. An F value of 0.66 with four degrees of freedom leads to a significance of only 0.618. Thus, the null hypothesis concerning the interaction of water temperature and detergent concentration cannot be rejected. In 1980, Janecek et al. also found no significant interaction of the same two variables when they studied the survival of Escherichia coli at low water temperatures.

Interaction of Water Temperature, Detergent Type, and Detergent Concentration

At 60°C, the only instance in which fungi survived on the fabric after laundering with detergent type Tide was at the high detergent
concentration. Three detergent types, Tide, Wisk, and Biological Bold, had a complete fungal decontamination at a water temperature of \(60^\circ C\) with low detergent concentration (see Table 6).

The highest water temperature utilized in this study, \(60^\circ C\), accounted for the greatest reduction of contaminated fabric swatches with four of the six detergent types. Other authors, such as Manikowske (1977), Marshall (1971), and Khan and Riggs (1980), stated that water temperature was the most important factor in reducing fabric contamination. In this study two exceptions are to be found when comparing the results obtained after laundering with detergent types Oxydol and Wisk. For Oxydol, decontamination was superior when laundering at water temperature of \(51^\circ C\) and low detergent concentration compared to results obtained after laundering with a water temperature of \(60^\circ C\) and high detergent concentration. The second exception is with detergent type Wisk. For this detergent type, better decontamination occurred when laundering at water temperature of \(51^\circ C\) with the high detergent concentration compared to decontamination after laundering at water temperature of \(60^\circ C\) with the high detergent concentration. Such relationships are indicated by the data from Table 6 and Table 7. Low water temperature of \(43^\circ C\), regardless of the detergent type and the detergent concentration, did not contribute at all to discernible fungal destruction.

Another significant interaction resulted for water temperature, detergent type, and detergent concentration as shown in Table 5. The \(F\) value is 4.36 with a degree of freedom of 20 which gives a significance less than 0.001. The null hypothesis concerning the interaction
### Table 6
Mean Number of Contaminated Swatches
After Laundering at 60°C (N = 54)

<table>
<thead>
<tr>
<th>Detergent Type</th>
<th>Detergent Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Tide</td>
<td>0.00</td>
</tr>
<tr>
<td>Oxydol</td>
<td>0.33</td>
</tr>
<tr>
<td>All</td>
<td>1.67</td>
</tr>
<tr>
<td>Biological Bold</td>
<td>0.00</td>
</tr>
<tr>
<td>Wisk (USA)</td>
<td>0.00</td>
</tr>
<tr>
<td>Dynamo</td>
<td>0.33</td>
</tr>
</tbody>
</table>

### Table 7
Mean Number of Contaminated Swatches
After Laundering at 51°C (N = 54)

<table>
<thead>
<tr>
<th>Detergent Type</th>
<th>Detergent Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Tide</td>
<td>5.00</td>
</tr>
<tr>
<td>Oxydol</td>
<td>1.33</td>
</tr>
<tr>
<td>All</td>
<td>3.67</td>
</tr>
<tr>
<td>Biological Bold</td>
<td>5.00</td>
</tr>
<tr>
<td>Wisk (USA)</td>
<td>4.67</td>
</tr>
<tr>
<td>Dynamo</td>
<td>4.33</td>
</tr>
</tbody>
</table>
of water temperature, detergent type, and detergent concentration has
to be rejected since the effect of water temperature varies with deter­
gent type and with detergent concentration.
SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

This chapter summarizes the study, presents the main results, and makes recommendations for further study.

Summary of the Study

The problem investigated in this study was the effect that various combinations of six detergent types, of three detergent concentrations, and of three water temperatures have on sanitation of fabric. The objectives of the study were the following:

1. To determine the effect of water temperature on survival of the fungus *Trichophyton mentagrophytes*.
2. To evaluate the importance of detergent type on survival of the fungus *Trichophyton mentagrophytes* on fabric laundered at various water temperatures.
3. To investigate the role of detergent concentration on survival of the fungus *Trichophyton mentagrophytes* on fabric laundered at various water temperatures.
4. To examine any interaction between type and concentration of detergent, and different water temperatures on the survival on the fungus *Trichophyton mentagrophytes* on fabric.

Three water temperatures, 43°, 51°, and 60°C, were selected for this study since they are representative of those chosen by consumers for home laundering. The detergents were chosen on the basis of retail
availability and distinctive components in their formulations. Surfac­
tants and builders differ as well as additives, such as oxygen bleach
and enzyme. The detergent concentrations chosen corresponded to half
the manufacturer's recommendation, the exact recommendation, and twice
the recommendation.

A typical 50% polyester/50% cotton blend sheeting
fabric with a durable press finish was chosen for the laundering test.
Sterile fabric specimens were inoculated with a broth culture of the
fungus Trichophyton mentagrophytes. Inoculated fabric specimens were
laundered in a top-loading heavy duty home washer. A ten-minute
regular wash cycle was utilized.

After laundering, fabric swatches were cut aseptically and depos­
ited on RODAC plates. Contaminated fabric swatches were counted to
determine fungal survival after laundering at various conditions. The
data derived from RODAC counts were statistically analyzed using the
analysis of variance procedure. This test specified which variables and
their interactions had significant effects on the number of contaminated
fabric swatches after laundering treatments. The results found here
confirm the fact that textile materials can provide an adequate environ­
ment for growth of microorganisms such as Trichophyton mentagrophytes.

Considering removal of Trichophyton mentagrophytes, the results
of this study revealed that water temperature is the most significant
variable among those selected for the study. Values in Table 8 show the
prime importance of water temperature, especially the one at 60°C at
which fungicidal effects were most evident. Fabric samples laundered
in water temperature of 51°C and 43°C had high fungal survival rates;
therefore, low water temperatures cannot be recommended for eliminating fungi capable of causing disease. The significance of the fungicidal effect of water temperature was less than 0.001 which is highly superior to the probability level of 0.05 accepted for this study.

The variable detergent type had a significant effect on fungal survival. It was the second most important variable after water temperature (see Table 8). The detergent type Wisk, with a citrate builder, was more effective than water alone or than any of the five other detergents. No direct conclusions could be drawn about the fungicidal effect of the additives—oxygen bleach and enzyme—since the detergent types containing one of these two additives were no more fungicidal than the other detergent types. The number of contaminated fabric swatches obtained using All, Biological Bold, Dynamo, or Oxydol detergents were similar to those where no detergent was added.

Wisk and Tide, the best performers, were more efficient when the manufacturer's recommended detergent concentrations were used. For the other detergents, a better performance was obtained when higher and lower detergent concentrations were tried. When the mean number of contaminated swatches was considered at each detergent concentration, fungal survival was higher at the high detergent concentration. The difference was not significant; thus the null hypothesis concerning detergent concentration was supported by the findings of the study.

A statistically significant difference in the variance of fungal survival was found to exist between the interaction of water temperature and detergent type. The interaction of detergent type and detergent
Table 8
Category Means for Each Variable Expressed as Deviations from the Grand Mean 3.20

<table>
<thead>
<tr>
<th>Independent Variable and Category</th>
<th>n</th>
<th>Unadjusted and Adjusted Deviation</th>
<th>Eta and Beta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detergent Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tide</td>
<td>27</td>
<td>-0.16</td>
<td></td>
</tr>
<tr>
<td>Oxydol</td>
<td>27</td>
<td>-0.01</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>27</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Biological Bold</td>
<td>27</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Wisk (USA)</td>
<td>27</td>
<td>-0.68</td>
<td></td>
</tr>
<tr>
<td>Dynamo</td>
<td>27</td>
<td>0.17</td>
<td></td>
</tr>
</tbody>
</table>

Detergent Concentration

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>54</td>
<td>-0.07</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>54</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>54</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

Water Temperature

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>60°C</td>
<td>54</td>
<td>-2.57</td>
<td>0.16</td>
</tr>
<tr>
<td>51°C</td>
<td>54</td>
<td>0.77</td>
<td>0.02</td>
</tr>
<tr>
<td>43°C</td>
<td>54</td>
<td>1.80</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Multiple R Squared: 0.785 or 78.5%
concentration was also significant even if no significant difference was found on the basis of the variable detergent concentration alone. The null hypothesis concerning the interaction of water temperature and detergent concentration was supported by the findings of the study.

**Conclusions**

The following conclusions can be drawn from the research findings:

1. The number of contaminated fabric swatches was reduced from 100 to 79.2% by raising water temperature from 43°C to 51°C; on raising water temperature to 60°C, however, contamination was reduced to only 12.6%.

2. The detergent type had an effect on fungal survival. Wisk was best in reducing survival. Differences among detergents were clearly evident only at the intermediate water temperature, 51°C.

3. The difference in fungal survival between laundering with or without detergent was significant only at the intermediate water temperature.

4. The detergent concentration had no significant effect on fungal survival in considering the data as a whole. However, with Wisk and Oxydol a relationship between fungal survival and detergent concentration was exhibited at the intermediate water temperature.

5. Fungal survival varied significantly with the chosen detergent types only at the intermediate water temperature.
6. The overall interaction of detergent concentration and detergent type was significant. Most of this effect was attributable to the detergents Wisk and Oxydol at the intermediate water temperature.

7. The overall effect of water temperature varied significantly with detergent type and with detergent concentration. However, no dependence was found at the low water temperature.

The results of this study confirm that to obtain a sterile textile product it is absolutely necessary to launder in water temperatures above 60°C, regardless of detergent type and of detergent concentration. In all cases where it is not possible to select such a high water temperature the addition of biocides in the detergent formulations should be considered.

Laundering in low water temperatures contributes to energy conservation which is the reason why consumers select mainly lower water temperatures. Nevertheless, the results obtained prove that complete sanitation is not guaranteed. However, it is possible to obtain a certain degree of sanitation when laundering in water temperatures between 51°C and 60°C with selected detergent formulations. In this study the detergent Wisk, whose builder is sodium citrate, was particularly adequate even at 51°C in terms of decontamination.

Recommendations

Further research needs to be done, for example, in the use of disinfectant in combination with low water temperatures to determine
whether chemicals may compensate for a reduction in water temperature. To confirm the influence of the variable detergent type on fungal destruction other detergent formulations should be investigated. Continuing study is needed since detergent formulations change continually.

Studies concerning cross-contamination when laundering in home washers and especially in communal facilities would be beneficial to consumers. Clearly this is a potential danger.

It would be interesting to investigate the effects of fiber content, of fabric construction, and of fabric finishes such as flame retardants and biocidal finishes. These may influence the susceptibility of the fabric to microbial attack or its removal and transference during laundering.

Organisms other than the fungus *Trichophyton mentagrophytes* might be investigated to discover if the conclusions concerning the fungicidal effect of the detergent types selected for this study are the same for other microorganisms such as bacteria and viruses. Also, another area of research is the determination of consumer knowledge concerning the influence of variables capable of causing disease mainly when communal facilities are utilized for laundering.

These suggestions are a few of the numerous research possibilities in a field that is important since it relates closely to health. An overemphasis on economy could constitute a threat to the public.


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APPENDIX A

LETTERS
March 22, 1983

Ms. Georgette Durand
Ecole de nutrition et d'études familiales
Université de Moncton
Moncton, N. -B
E1A 3E9
Canada

Dear Professor Durand:

We have received your letter inquiring if Lever manufactures any detergents containing enzymes.

I have discussed your question with our research people and have been advised that we do not manufacture enzyme detergents.

We appreciate your interest in Lever Brothers Company and hope that the above information will be helpful.

Sincerely,

Nancy Scott
Consumer Service Department

NS:fw
April 7, 1983

Ass. Prof. Georgette Durand  
Ecole de nutrition et d'études familiales  
Université de Moncton  
Moncton, Nouveau-Brunswick, Canada E1A 3E9

Dear Ass. Prof. Durand:

This responds to your recent request for information concerning Procter & Gamble detergent products which contain enzymes.

We currently market two national brands containing enzymes: Biz all fabric bleach and Era Plus heavy-duty liquid laundry detergent.

We hope this information is helpful. Thank you for writing.

Sincerely,  

[Signature]

Patricia M. Jent

Attachment  
6041P
May 11, 1983

Ms. G. Duran
Ecole de Nutrition et d'etudes familiales
Universite de Moncton
Moncton, N.-B.
E1A 3E9

Dear Ms. Durand:

Thank you for taking the time to write about products.

Colgate-Palmolive has always found the opinions of our consumers to be a most helpful way of measuring the success of our products. Your reaction to our brands is the best way for us to better understand your needs and desires. To answer your question, Axion, Fresh Start, and Dynamo Action Plus contain enzymes, as you can see in the enclosed ingredients lists. I'm sorry to disappoint you, but we cannot release confidential information regarding our markets with these products.

We certainly appreciate your comments and will welcome hearing from you at any time, whether in praise or criticism.

Very truly yours,

Cheryl Pryor
Consumer Specialist

pt
Dynamo Action Plus Ingredients

Water
Ethoxylated Alcohol
Sodium Alkylbenzene Sulfonate
Ethyl Alcohol
Triethanolamine
Sodium Formate
Enzyme
Brighteners
Color
Perfume
### Fresh Start Ingredients

<table>
<thead>
<tr>
<th>Phosphate</th>
<th>Non Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Tripolyphosphate</td>
<td>Sodium Aluminum Silicate</td>
</tr>
<tr>
<td>Alkoxylated Alcohol</td>
<td>Alkoxylated Alcohol</td>
</tr>
<tr>
<td>Sodium Silicate</td>
<td>Sodium Carbonate</td>
</tr>
<tr>
<td>Water</td>
<td>Sodium Bicarbonate</td>
</tr>
<tr>
<td>Alkaline Protease</td>
<td>Sodium Silicate</td>
</tr>
<tr>
<td>Brightener</td>
<td>Water</td>
</tr>
<tr>
<td>Color</td>
<td>Brightener</td>
</tr>
<tr>
<td>Perfume</td>
<td>Enzyme</td>
</tr>
<tr>
<td></td>
<td>Perfume, Color, etc.</td>
</tr>
</tbody>
</table>
Axion Ingredients

- Sodium Sulfate
- Sodium Tripolyphosphate
- Water
- Sodium Alkylbenzene Sulfonate
- Sodium Silicate
- Alkoxylated Alcohol
- Alkaline Protease
- Perfume and other minor ingredients
Professor Georgette Durand  
Ecole des sciences domestiques  
Centre universitaire de Moncton  
Moncton, New Brunswick  
E1A 3E9

March 9, 1981

Dear Professor Durand:

Thank you for your enquiry of February 26 about the products of our company. I have listed our main products in the following and have shown the builders and actives used in each.

Concentrated 'all':
Active - nonionic
Builders - phosphate, NTA, carbonate, silicate.

Surf, Breeze, Sunlight:
Active - anionic
Builders - phosphate, NTA, carbonate, silicate.

Amaze:
Active - nonionic
Builders - phosphate, NTA, carbonate, silicate
Contains enzymes.

Wisk (liquid):
Active - mixed nonionic and anionic.
Builder - NTA.

You will find that nearly all detergents sold in Canada contain 5% phosphate (expressed as $P_2O_5$) which is the maximum permitted by law. I am not aware of any products in Canada which contain citrate. I believe aluminosilicate is used only by one brand in test market in Western Canada.

The other builders listed are in general use in most nationally distributed brands.

Strictly speaking Amaze, our enzyme brand, should not be listed as a detergent. It is recommended for use as a pre-soak and as an additive to the detergent system. However it does contain all the essential components of a detergent and will function very well in that application.

I trust the above information will be helpful to you in your studies relating to detergents.

Sincerely yours,

[Signature]

K. Clark
Research & Development Manager

lm
March 9, 1981

Prof. Georgette Durand  
Ecole des sciences domestiques  
Centre universitaire de Moncton  
Moncton, N.B.  
E1A 3E9

Dear Professor Durand:

Thank you for writing to us concerning the surfactants and builders used in our laundry detergents.

The surfactant system of our main detergents, Tide, Cheer, Oxydol, and Bold, is based on LAS (linear alkyl sulfonate).

Our detergents are of course restricted by law to 5% complex phosphates with the balance of the builder made up of NTA (sodium nitrilotriacetate) in our four detergent brands. Our research has shown citrates, carbonates and silicates to have some disadvantages when used as the main builder component.

Naturally, since the state of detergency technology can change dramatically in a short time, we are always testing new formulations and new ingredients. Some areas of the country may be used to test product variations, so the nature of our surfactants and builders discussed above relates specifically to the Maritime provinces at this point in time. The situation could be different a year from now.

I am also enclosing two bulletins which we send to students and consumers who ask us these questions. They may be of additional help.

Sincerely

(Ms.) Ann E.M. Echlin  
Supervisor, Educational Services

Enclosures
Table A
Number of Contaminated Fabric Swatches After Laundering with Six Detergents, Three Detergent Concentrations and at Three Water Temperatures

<table>
<thead>
<tr>
<th>Detergent Concentration</th>
<th>Tide</th>
<th>Oxydol</th>
<th>All</th>
<th>B.Bold</th>
<th>Wisk</th>
<th>Dynamo</th>
</tr>
</thead>
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<td><strong>Laundering at a water temperature of 60°C</strong></td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
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<td>1</td>
<td>0</td>
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<tr>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Medium</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
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<td>0</td>
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</tr>
<tr>
<td>High</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>1</td>
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<td>1</td>
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<td><strong>Laundering at a water temperature of 51°C</strong></td>
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<td>2</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
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<td>4</td>
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<td>2</td>
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<td>5</td>
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<td>5</td>
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<tr>
<td>Medium</td>
<td>3</td>
<td>5</td>
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<td>4</td>
</tr>
<tr>
<td>1</td>
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Table A (continued)

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<th>Detergent</th>
<th>Detergent Type</th>
<th>Tide</th>
<th>Oxydol</th>
<th>All B.Bold</th>
<th>Wisk</th>
<th>Dynamo</th>
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<tbody>
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<td>Concentration</td>
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<tr>
<td>Low</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>2 5 5 5 5 5 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 5 5 5 5 5 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Medium</td>
<td>1 5 5 5 5 5 5</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>2 5 5 5 5 5 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>3 5 5 5 5 5 5</td>
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<td></td>
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</tr>
<tr>
<td>High</td>
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<td>2 5 5 5 5 5 5</td>
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<td></td>
<td>3 5 5 5 5 5 5</td>
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<td></td>
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</tbody>
</table>

*aMaximum contamination equals 5

*bThe numbers 1, 2, and 3 correspond to each replica
Table B
Number of Contaminated Fabric Swatches After Laundering Without any Detergent

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Water Temperature</th>
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<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
</tr>
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