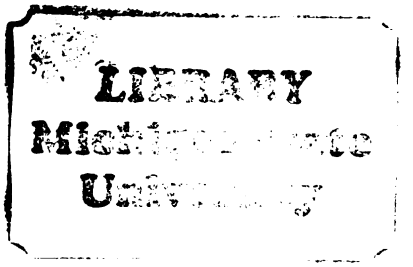

This is to certify that the

thesis entitled

ELECTROSTATIC ATTRACTION OF MICROORGANISMS TO POLYSTYRENE

presented by

STEVEN B. LYMAN

has been accepted towards fulfillment
of the requirements for

M. S. degree in PACKAGING

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ELECTROSTATIC ATTRACTION OF MICROORGANISMS TO POLYSTYRENE

By

Steven B. Lyman

A THESIS

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ABSTRACT

ELECTROSTATIC ATTRACTION OF MICROORGANISMS TO POLYSTYRENE

By

Steven B. Lyman

Sterile polystyrene petri dishes were used as a model to examine whether electrically charged plastic containers attract airborne microorganisms. Negative and positive charges were induced on the dishes while the control sample had no charges. The experiments were carried out in an open air environment to simulate "real world" conditions.

The results of the tests indicate that positively charged petri dishes usually attract about twice as many airborne microorganisms than either the negatively or zero charged plastic dishes. The negatively and zero charged dishes had similar bacterial counts. These studies revealed that microorganisms, because of their negative charge or adherence to negatively charged airborne particles, are attracted to positively charged polystyrene containers. There is a definite need for sterile containers to maintain negative or zero charges.

DEDICATION

This thesis is dedicated to my parents, Richard and Irma Lyman, without whose support and love this thesis would not have been possible. To Carol Rewers whose love, understanding, and consistant encouragement helped me over the rough times.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

In a recent experiment performed by J. Kabara, bar soap in public lavatories was found to contain high levels of microorganisms (19). This work suggested that a possible method of transmitting infection from one individual to another is through the use of the same bar soap. In the case of the liquid soaps tested, which were housed in plastic containers, the soap showed no sign of contamination. However, the outside of the liquid soap container did contain organisms.

Nosocomial infections are a major concern for most hospitals since many infections could be prevented by proper handwashing (19). The use of plastic soap dispensers could reduce the spread of disease in hospitals and the home. The introduction of plastic soap dispensers may decrease the probability of infection through contact, because the amount and length of time in contact with a surface is much shorter when using a liquid soap dispenser as compared to handling a soap bar.

Over the years the utilization of plastic has steadily been increasing in all types of container materials (27). Its light weight, low cost, durability, and ease of forming any shape or size make it the container of the future. A

good example is the increased application of plastic materials in hospitals and the pharmaceutical industry.

A question has arisen concerning the maintenance of sterility for the product and package with these new plastics. All signs indicate that there is no problem, but there are several questions which have arisen. Plastics in general are insulators so they charge easily when rubbed with other materials. How does this electrostatic charge affect a sterile product/package? Does the electrostatic charge attract microorganisms and if so, how? These are the questions which this research set out to answer.

CHAPTER 2

ELECTROSTATIC CHARGING

History

The Greek philosophers, who theorized that matter was made up of tiny particles or atoms, included an explanation of the phenomenon we now call electrostatic attraction (20). The matter received no serious investigation until in the sixteenth century, William Gilbert of England performed a detailed investigation into the attraction of substances. This was the starting point for many other scientists throughout Europe. In 1747 Benjamin Franklin gave the name "positive" to rubbed glass, and "negative" to rubbed resign. Other scientists involved include Michael Faraday who introduced the concept of line of force surrounding charged bodies, and Robert J. Van de Graaff inventor of the electrostatic generator (23).

In the Environment

Static electricity is ubiquitous. It can be seen in one of its most lethal forms during a thunder storm as lightning bolts. Another common example is the slight shock you receive during the winter after walking across a carpet and touching an object. Other occurrences of static electricity are a major problem and can often be dangerous, such as the detonation of explosive dust in a grain elevator or gases in

a hospital operating room (30). These are real problems and there are rules and regulation governing the operating procedures of these facilities.

Static electricity can also be a nuisance in plastic plants and paper and textile mills, causing material to stick together and jam machinery. Another problem that has received little attention results from the attraction of charged objects for dirt and foreign debris (15). This is a concern if a product/package sits on a shelf and attracts dust, making it unattractive for sale.

Electrostatic discharge (ESD), the discharge of static to or from computer chips or intergrated circuits (17), has been recognized since the early 1960's, but no major breakthrough in the elimination of ESD has yet to come about. With the increase miniaturization of IC units, less ESD voltage is required for damage, so ESD plays an ever increasing role within the electronic industry, causing millions of dollars in damage each year (23).

Theory Behind Static Charge

When two objects are brought together into contact, the free electrons at the surface may be transferred back and forth. When the two materials are separated, one material will retain more of the electrons and be negatively charged, while the electron deficient material is left positively charged (20). The amount of charge transferred is a function of pressure and velocity of contact. Greater pressures of contact produces an increase in friction and makes it

easier for electrons to transfer, thus increasing the charge. Increasing the speed of separation increases the charge imbalance because there is less time for the two objects to rebalance their charges (30).

Methods of Generating Charge

Static charge can be developed in a number of ways. Every motion or separation between objects or exposure to electrostatic fields, and charged particles, can cause an object to become charged. Many factors from the conductivity of the material to the relative humidity will effect how great a charge is produced (11).

In contact charging, electrons are transferred from one material to the other. In this case no movement is necessary. The amount of charge transferred is dependent on the materials being used. Electrons will move more freely if the materials are both conductors. If the materials happen to be insulators, as in most plastics, only electrons on or close to the contact surfaces transfer. The net charge transfer depends on the surface properties of the materials in contact (30).

Triboelectric charging is probably the most common type of electrostatic charging. Tribo means rubbing; triboelectric charging is the rubbing together of two substances. Contact charging and triboelectric charging are similar in that contact between substances must be made. Triboelectrification occurs when two substances moving relative to each other are brought together and a charge is generated.

The amount of this charge is dependent on several factors (3) like contact pressure, speed of rubbing, and smoothness of the surfaces (23).

If an uncharged object is brought within the electrostatic field of a charged object, the uncharged object will become electrically polarized. The surface of the object closest to the charged object will have an equal and opposite charge, and the furthermost surface, will contain the like charges. Bleed-off of the charge from one of the surfaces will result in a charge imbalance. This is known as induction charging. The main difference between this and contact charging is that this method requires no physical contact between objects to obtain a charge (23).

Charged particles from radiation and electron beams which come in contact with surfaces of a material can impart a charge on it. Ion and electron beam charging occurs when electrons collide with the surface of the material and fill the outer electron orbits of the atoms near the surface of the material producing a negatively charged object.

Properties of Plastics

Plastics are organic materials made of large, long chain polymer molecules. Properties of various plastics depend on the size of these long chain molecules and on the arrangement of molecules within the chain. The versatility of these polymers is one of the major advantages of plastics. Some plastics have excellent optical properties, others high strength, and all are free from atmospheric

corrosion, a characteristic of no other packaging materials (14).

In this research petri dishes made of polystyrene, a very commonly used plastic, were used. It is a rigid, hard thermoplastic with the attractive ability to blend with other materials and form plastics with a large number of different properties. Although brittle, polystyrene products are clear with good appearance. Because of its good electrical properties, (high surface resistance) this type of plastic is sometimes used as electrical insulator (6).

Polymers in general are excellent electrical insulators, and display high electrical resistance and very little electrical conductivity. Because they are inherently insulators, they are more susceptible to accumulation of static electricity.

A polymer's electrical properties are usually not dependent on its molecular weight and can be strongly affected by the addition of plasticizers. The observed conductivity in plastics is most likely due to electrolytic motion of ionic impurities within the polymer. If there is a decrease in electrical resistivity, it is largely caused by both ionic impurities and mobility. It is fairly difficult to determine ionic impurities but mobility is easily measured by mechanical and thermal properties. The addition of a plasticizer to a polymer causes a decrease in electrical resistance, but the relationship is not directly proportional to the amount of plasticizer added (33).

Most plastics when brought into contact with other materials such as metals will become negatively charged. Figure 1 contains the triboelectric series, a method of showing the charging relationships among certain chargable materials. Cotton is used as the reference material, and since it tends to absorb moisture it is somewhat conductive and when rubbed against another material cotton tends to induce a static charge. Materials listed above cotton acquire a positive charge (giving electrons off) when rubbed with cotton, and those below a negative charge (accepting electrons). In the case of two plastics being rubbed together the material highest on the table will charge positively and the lower, will charge negatively (32).

Moisture in the atmosphere plays a key role in the static electrification of plastic. An increase in relative humidity lowers the voltage at which air breakdown occurs and allows discharge/sparks to be produced more readily. With increasing relative humidity, water vapor will absorb on the surface of a material and the water molecules will diffuse into the surface, increasing conductivity (30). If a large volume of air is drawn across the surface, the humidity level on the surface will decrease. The lower moisture level at the surface reduces the conductivity of the surface and increases the static charge on the surface (26). Under high humidity conditions, leakage rate or the slow static discharge of a material is increased, but in low humidity, high static buildup can be developed. Figure 2 contains examples of low and high humidities and voltages

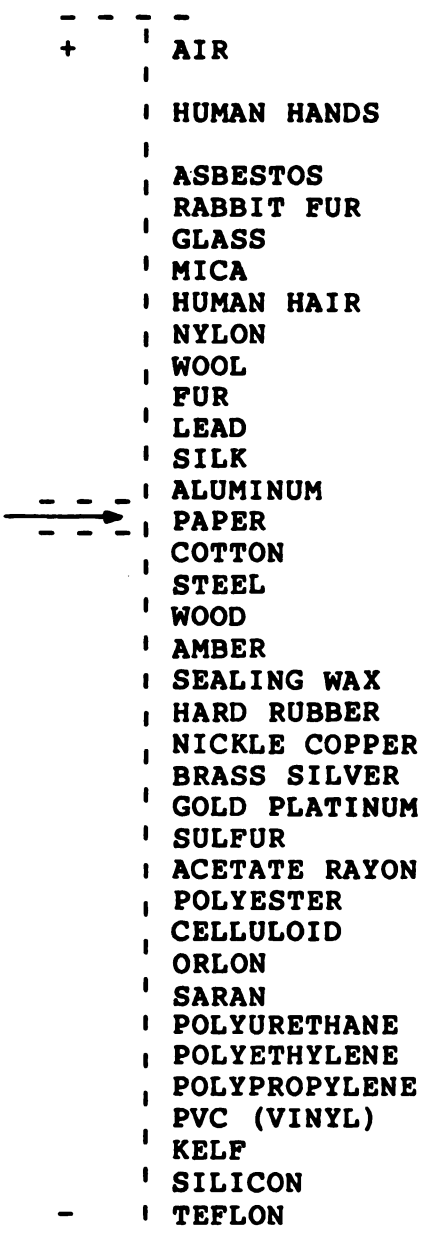


Figure 1. Tribroelectric series (32).

Means of Static Generation	Relative Humidity	
	Low, 10-20%	High, 65-90%
Walking across carpet	35,000 V	1500 V
Walking over vinyl floor	12,000 V	250 V
Worker at bench	6000 V	100 V
Vinyl envelopes for work instruction	7000 V	600 V
Common poly bag picked up from bench	20,000 V	1200 V
Work chair padded with urethane foam	18,000 V	1500 V

Figure 2. Typical Measured Electrostatic Voltages (31).

generated during different moving operations. Note that static charge can still be generated at high humidity, but at a much lower rate.

Additives & Treatments to Plastics

Plastics are seldom comprised entirely of just polymer molecules. They usually include various other ingredients such as filler, pigments, lubricants, plasticizers, humectants, and antistatic liquids, most of which help to minimize static electrification (6). Plastics which are nonconductive usually have a resistivity range from 10^{14} ohm/square inch to 10^{16} ohm/square inch. The most effective way to reduce this and make the material less prone to static electricification is with conductive fillers, internal antistats, and external antistats. Each has slightly different advantages and disadvantages but all are adequate for reducing static charge (21).

Conductive fillers or reinforcements are any conductive or metallic material added to the plastic that eliminates static. The main process for electrical conduction through a conductive plastic is by particle-to-particle contact. Typical conductive fillers are carbon black, aluminum flakes or fibers and polyacrylonitrile carbon fiber. The major advantage in using fillers is the permanent conductivity they impart. The effect does not rely on the moisture content of the surrounding atmosphere. The disadvantage is the resulting black or gray color of the plastic material (5).

Internal antistatic agents are compounds which are incorporated into the bulk polymer during or just prior to processing. They usually consist of either ionic organic salts, alkylamines, hygrophilic organic compounds, or polyethoxylated glycolester (9). Once in the bulk polymer they migrate to the surface and attract moisture from the surrounding air, producing a thin layer of water which helps to dissipate static charge. The main consideration in using this method is the compatibility with the main polymer and the rate of migration. For these reasons not all polymers can use internal antistats. Those polymers in which internal antistats are effective are Polyethylene, Polypropylene, Polyvinyl Chloride, and Acrylonitrile-Butadiene-Styrene (21). The advantages of the internal antistat are the low cost, ability to be colored, and transparency.

External or topical (surface) antistatic agents are generally liquids which are applied in a secondary operation to the surface. These usually consist of two components; a carrier and an antistat. The carrier, consisting of either water, alcohol, or other solvent, controls the amount of antistat applied to a surface. The antistat may be composed of quaternary ammonium compounds, amines, phosphate esters, and polyhydric alcohol like glycerine and sorbitol (3). The topical solution reduces friction by increasing lubricity on the surface, as well as increasing conductivity and may be used on almost any material.

External antistats are applied to the finished product by either spraying or dipping. External antistats like the

internal antistats are dependent on a surface moisture layer to provide a means for static elimination. The main disadvantage is that they are easily rubbed, wiped or rinsed off leaving the surface non-antistatic. The external antistats are probably the least costly means of static charge control and are compatible with all plastics and most other materials (15).

CHAPTER 3

AIRBORNE MICROORGANISMS

History

The first known experiments concerning the microbiology of air were done by Pasteur. He showed the presence of bacteria in the air in the early 1860's. In the late 1860's a method of antiseptic surgery was used by Lister. Using an atomizer to spray a fine mist of carbolic acid into the air, it was soon discovered that surface contact with organism, not just the presence of airborne bacteria, was the cause for infection (4).

Between 1897 and 1898, Professor Flugge determined that the air transmission of disease was of little importance. His conclusion was based on an experiment in which Petri dishes were exposed in front of persons who would sneeze or cough. He concluded that all infective droplets settled out within a few feet of the person and thus diseases were not airborne. Because of this experiment physicians dismissed the idea of airborne disease and little work was done in the area until World War I.

At the Harvard School of Public Health, William Well in 1933 using an atomizer, sprayed droplets of a bacterial suspension over a large area. The bacteria was recovered using a centrifugal air sampler. It was concluded that

droplets 0.1mm in diameter or larger did settle before evaporating. Smaller droplet evaporated in air leaving a nuclei which was still infective and could be carried indefinitely in air current (4). Since this time there has be a slowly growing interest in the microbiology of air.

Microbiology of Air

Air is considered an inert vehicle which can carry various bacteria, fungus spores and filaments, and virus particles. Air is not a medium in which microorganisms can grow but rather carries particulate matter, dust, and/or droplets on which microorganisms may ride. Air does not contain the necessary amount of moisture and nutrients in a usable form for bacteria and other microorganisms to grow. There are many sources which introduce microorganisms into air, but the main form are from dust particles containing dry vegetative cell and spores (28).

Different localities have somewhat varying species of organisms but certain forms are present throughout the world. The number of organisms in the air is dependent on the amount of activity in the environment and the amount of dust stirred up. An active environment will show higher organisms counts than an inactive one. It has been found that there are more bacteria in the air over fertile cultivated soil than over poor soil. Also the air over bare surfaces contain more organisms than air which is covered with vegetation (28).

Microorganisms are seldom found in a free state or by themselves in air. They are usually attached to some form of floating particles like dust, saliva, and carbon. Organisms in the air are slightly heavier than air and will settle out very slowly in a quiet atmosphere. They may be kept suspended indefinitely with a gentle current.

Some microorganisms can remain in air for long periods of time. This suspension time is dependent on the speed of the air current, the size of the particles of which they are attached, and the moisture or humidity of the air. Organisms which attach themselves to dust or droplets of moisture settle at a much faster rate. In a damp humid condition there are fewer organisms than a dry one, probably because the organisms are carried down by droplets of moisture. During dryer summer months there are more organisms in the air than in the wet winter months (28).

Organism survival is a function of both relative humidity and temperature. Organisms once introduced into the air may die in a matter of seconds, or may survive for weeks, months, or longer. The life of an airborne organism is dependent on many factors including atmospheric condition (humidity, sunlight, temperature), the size of the particle which the organisms is on, and the degree of susceptibility or resistance of the particular organism (25). As humidity increases and temperature decrease, airborne organism death rate decreases. Extremes in temperature increase the decay rate of most organism population, but for

humidity the inverse is true, with 40 to 80 percent humidity being less favorable for microorganisms (28).

Outside Environment

Dust is a product of wind motion against soil and this dust carries the microorganisms that are in the soil. Moisture and the droplets of water come from bodies of water and enters the atmosphere through evaporation and wind action. In the outside environment organisms are subject to many air currents, which can carry organisms great distances. The dispersion and survival of organisms over the earth's surface is influenced by a complex set of conditions, including time of day, season of year, and climatic conditions.

Bacteria and mold spores have been found high above the surface of the earth. They originate at the surface (soil or water) and are dispersed as high as 11,000 feet (4).

Inside Environment

The extent of microorganism contamination of indoor air is influenced by the ventilation rate, crowding, and the amount of activity within the confines. In large buildings there may be considerable air movement. Stairwells may act like smoke stacks with hot air rapidly rising to the top. Elevators act like plungers when moving up and down (4). Both will cause air and organism movement.

Dust on floors and other surfaces become airborne during periods of activity. Since microorganisms survive for long periods in dust, stirred air creates significant problems in hospitals and other institutions wherever crowds are

gathered. The bacteria content of room air under several different conditions is shown in Figure 3 (10).

Techniques for Air Analysis

Special apparatus are required for the sampling of air to determine organism content. Several devices have been developed for this purpose, including solid impingement devices where microorganisms are collected or impinged on the solid surface of an agar medium or filter disk. In liquid sampling devices air is passed through a broth or other liquid medium where the organisms are collected.

The Anderson air sampler consists of six circular collecting devices which holds six petri dishes filled with suitable agar medium and resembles a stacked sieve collector. The top collector plate has uniformly distributed large holes over the first petri dish, the second plate has slightly smaller holes and each following plate has progressively smaller holes. When air is drawn down in through the stack the large particles are deposited on the top plate and the smaller particles are carried on by the stream of air to the lower dishes until it is unable to pass through the holes in a plate (25). This allows the particles to be automatically separated into six different sizes, large organisms at the top with decreasing sizes in the lower petri dishes.

The sieve and slit-type samplers are similar to the Anderson sampler. The sieve sampler draws a measured volume of air through a number of holes which are evenly

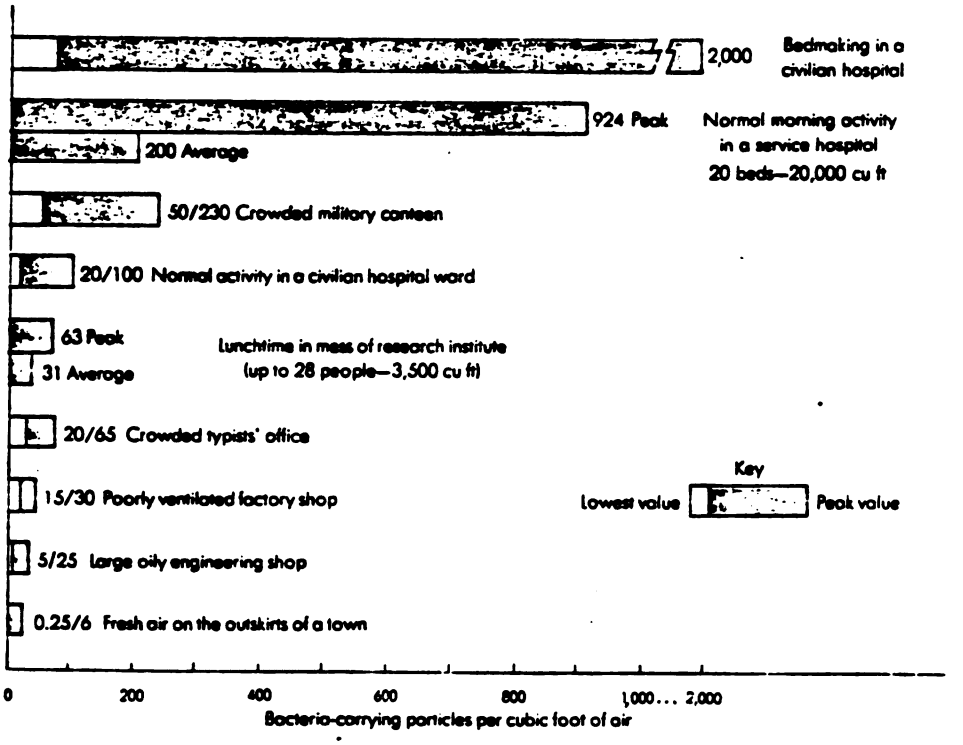


Figure 3. Bacterial content of air in civilian and military establishments. Measured using slit samplier (10).

distributed in a metal cover. Under the cover is the petri dish containing the agar medium. The air is drawn through and the particles are impinged upon the surface.

The slit sampler is more complex than the sieve sampler. Air is drawn in at high speed through a narrow slit onto the petri dish. The petri dish is rotated one complete revolution during sampling operation to obtain uniform distribution. The speed of rotation is adjusted according to the density of the microbial population. Air samples with high organism content have a higher speed than that for containing few organisms. This method has shown high efficiency in airborne organism recovery (25).

In the settling-plate technique, the method used in this research airborne organisms are collected on exposed agar surface for a period of time. The plates are then incubated and colonies counted. The method is very simple and only particles of certain size will settle onto the plate in a given time. The drawback of this method is that there is no way of knowing the volume of air actually sampled, nor does it indicate the number of organisms present in a given volume of air. Results can be influenced by air movement, so while the values obtained by this method are not quantitative the method does allow for relative comparisons. With repeated use for fixed time periods rough estimates of the extent of air contamination may be obtained (28).

Electrostatic precipitation samplers use an electrically charged surface to collect particles. Two

petri dishes are set on metal plates which are connected to an electrical source. The electrical source charges one metal plate positively and the other negatively. A blower draws air over the petri dishes (charged plates) at a constant rate (28). This concept was originally conceived because static charge is effective in collecting dust and smoke particles. Airborne organisms with a positive charge would be collected on the negative plate and those negative organism on the positive plate (7).

Results from previous tests show that an electrostatic charge greatly increases the collection of the organism Escherichia coli, with the distribution of organisms greatly affected by applied voltage and air rate. Positive electrodes collect more particles than the negative (22), and both negatively and positively charged plates collect more Escherichia coli plates than with no charge.

Sterilization and Disinfection

Controlling organisms in a particular room or building depends on the activities within. Hospital operating room air is unlikely to be sterile but airborne organism contact must be maintained at very low levels to reduce the chance of infection. Other areas of a hospital do not need as tight of control over airborne organisms but still should observe precautions and standard practices.

There are various methods available for reducing organisms in air. Ultraviolet radiation may be used to reduce airborne organisms. The germicidal lamps emit

wavelengths in the range of 250 to 260 nm, the most effive bactericidal region. This radiation has little penetrating power and will only be effective through direct contact with airborne organisms. Unlike visible light 250-260 nm uv radiation is not reflected throughout an area. The germicidal lamp also causes irritation to human skin and eyes, so great care should be taken when installing these lamps. Practical application may be direct irradiation of air with the germicidal lamp in areas which are unoccupied which allows the killing of any organisms. Another method may be indirect irradiating a room with the germicidal lamp in occupied rooms with the occupants shielded from the lamps rays. A third method is to irradiate the air before it enters a room in the air circulating system (4). This reduces the organism content of the air entering the room rendering the room virtually airborne-organism free.

A relatively recent innovation in the control of airborne organisms is the laminar-airflow system. This is used for closed space and has a unidirectional airflow. Air is passed through high efficiency particulate air (HEPA) filters made of cellulose acetate. This system is highly efficient in removing particles as small as .3 m and is now used in a variety of designs such as horizontal or vertical laminar flow clean rooms. One of the major uses of this system is in the electronics and aerospace industries and this technique may soon become a standard for the pharmaceutical and food industry (25).

The use of chemicals in the reduction and elimination or organisms has been widely used and studied. Their use in reducing airborne organisms is limited because contact is required to be effective. They are most effective when applied to surfaces but can be sprayed or atomized if needed.

Substances or chemicals which inhibit or kill microorganisms may be classified in two groups. Bacteriocidal agents will both inhibit growth and kill the microorganisms. Bacteriostatic agents will only limit the growth of microorganisms (12).

Time, temperature, concentration, and pH are several factors influencing the effectiveness of bacteriocidal and bacteriostatic agents. The more concentrated the bacteriocidal agent, the more quickly it will destroy an organism up to a certain concentration, the killing effect being nonlinear. No chemical agent, either bacteriocidal or bacteriostatic, acts instantly but all require time for contact, and allowing for the chemical or physical action to occur (12).

CHAPTER 4

TEST PROCEDURE

To determine the effects of charge on the attraction of airborne microorganisms to polystyrene petri dishes the research was broken into two phases. In phase one petri dishes containing agar and having a positive, negative, or zero charge were exposed to open air. The second phase was identical to the first except that the petri dishes were first sprayed with a topical (or surface) antistatic solution before exposure.

Using the open air cell or settling plate technique, the petri dishes were exposed to air in a room for a period of one hour. Particles or microorganisms settled on the exposed plates. After exposure the plates were incubated for microbiological evaluation (see techniques for air analysis). This exposure method was chosen because it represented real world conditions. Also, the exposure method was identical to earlier work done with bar and liquid soap which were left in a bathroom and later sampled for microorganisms (19).

There are several drawbacks to using this method. Results obtained have little quantitative significance but do provide comparative results or estimates. Also the quantity and size of the particles settling on the plates are

affected by air movement. This was overcome by controlling air movement and by using a large number of samples to adjust for variations between experiments.

For both phases the petri dishes were exposed to room air and immediately were charged positively or negatively (the control dishes were not changed). In the second phase the Staticide® topical solution was sprayed immediately before exposing the plates to air and then the charge was added. All procedures preceeding and following the spraying of the topical solution were identical to those used in the first phase.

The environmental conditions under which the experiment was run were the same for both phases. The tests were conducted at Michigan State University, in the School of Packaging, which house administrative and laboratory facilities. The mens lavatory was selected because similar conditions were used in a earlier work on bar soaps (19). The air in the lavatory was circulated by a floor fan to maintain constant movement.

The petri dishes were placed on a table roughly three feet above the floor's surface. On this table was placed a large flat slab made of an insulating material to prevent a charge leakage from one petri dish to another. The petri dishes were placed far enough away from metal objects to limit any chance of induction charging. The dishes were arranged randomly to prevent clustering of like charged dishes and to assure that the three different charges would be exposed equally to various air currents.

The length of time for each test exposure was one hour. This time was selected because the charge on the petri dishes would start to reduce or bleed-off after one hour. Earlier tests were run for two hour time periods in air, but did not result in a significant increase in colony forming units (CFU) over those exposed for one hour. Difficulty in maintaining the charge on the dishes was also experienced during the two hour test intervals.

After exposure the petri dishes were placed in a incubator at 37^{o}C for forty eight hours and then the colony forming units (bacteria) were counted.

Materials

Each test sample consisted of a petri dish containing agar. The dishes were made of polystyrene to simulate plastic container performance. Another benefit for using polystyrene is its excellent insulating properties allowing it to readily accept and hold a charge.

The agar used in these samples was Brain Heart Infusion (BHI) with 2% Tween 80 (polysorbate 80), a chemical neutralizer. Agar is a fairly conductive substance and will disperse a charge over its surface equally. This is known from past research using agar broth and electrical currents (19).

Petri dishes were charged with a Zero Stat® Gun. When the trigger of this gun is squeezed positive ions are emitted, while releasing the trigger forms negative ions. The ions emitted are generated by squeezing a peizo electric

crystal. Ion discharge was verified using test equipment which measures polarity, at the Chemistry Electronics Lab, Michigan State University. (The normal purpose of this commercially available gun is to shoot ions to clean phonograph records of static charge and dust). The samples tested were charged either to 500+ volts positive, or 500+ volts negative. Some samples received a charged greater but accuracy was limited to roughly 500 volts.

An electrostatic voltmeter was used to measure both the polarity and the amount of charge on each dish. The voltmeter was an ACL Model 300 Electrostatic Locator (1). The major advantages of this self contained meter was its light weight portability, allowing the experiments to be run anywhere. The meter has two operating ranges, 0-500 volts at a range of 0.5 inches, and 0-3000 volts at 4 inches (1). In our experiments, accuracy above 500 volts was extremely limited, because the nickel plated sensing electrode operates on a angular field of view, so distance from the charged surface must be accurately measured. Because petri dishes are fairly small, 2.5 in in diameter, the distance at which the meter could be held was approximately 0.5 inches. Readings were taken every 10 minutes to insure that the charge was maintained. If the charge was found to be reduced, additional charge was added using the Zero Stat Gun.

The second phase of our research was an investigation of the effect of Staticide application to the charged petri dishes. Staticide® is a proprietary water based compound

consisting of several quaternary compounds (2). In addition to its bacteriostatic properties this product has antistatic properties. Exposed petri dishes were sprayed with this solution, and the same procedures were followed as for phase one.

CHAPTER 5

DATA AND RESULTS

Raw Data

The temperature and relative humidity were measured before each of the 20 tests were performed. Each of the 20 test included 9 petri dishes: 3 positively charged (P), 3 negatively charged (N), and 3 with zero charge (0). The following abbreviations were used:

P_A, P_B, P_C	to signify	Positive	charged	petri	dishes
O_A, O_B, O_C	"	Zero	"	"	"
N_A, N_B, N_C	"	Negative	"	"	"
S^*P_A, S^*P_B, S^*P_C	"	Positive	"	"	"
S^*D_A, S^*O_B, S^*O_C	"	Zero	"	"	"
S^*N_A, S^*N_B, S^*N_C	"	Negative	"	"	"

*Staticide^R Topical Surface Solution treated.

During each experiment the charge on each petri dish was measured every 10 minutes for a one hour time period. If a dish's charge was measured to be below 500 volts, more charge was added using the Zero-Stat® Gun. Following exposure, the dishes were incubated for 48 hours at 37°C and then the colony forming units were counted. There were 22 different types of microorganisms which for short hand purposes were labeled A to Z. The colony forming units found

in the exposed petri dishes were categorized as shown in Table 1.

The majority of colony forming units were observed to be in the first four categories, A through D. Organism types E through Z were found infrequently and are grouped together as "All Others."

Gram stains were taken on the organisms which were grown on Blood Agar. If the stain is absorbed by the organism, it may be considered gram positive, if not, gram negative, and if partially stained, gram variable. The designation of either positive or negative gram stain has nothing to do with the charge polarity of an organism. Gram-negative microorganisms are those which may considered disease causing agents.

The different organisms were next analyzed using a microscope to develop further parameters for designation.

Organism A: Extra large cocci (individual cells), gram variable, colonies were: tetrads, clusters, pairs.

Organism B: Medium cocci, gram positive, colonies were: clusters.

Organism C: Large cocci, gram variable, colonies were: tetrads, clusters, pairs.

To determine whether the three microorganisms are truly gram-positive, or gram-negative, or yeast and fungi, the different organisms were plated on three different agars; McConkey agar allows only gram-negative organisms to grow,

Table 1. Description of Each Microorganism Type Found in Exposed Petri Dishes

A	Colony Size	Form	Elevation*	Margin*	Pigment*
A	Medium	Circular	Convex	Entire	Yellow
B	Large	Circular	Umbonate	Entire	White
C	Medium	Circular	Puluinate	Entire	Pinkish
D	Small	Circular	Convex	Entire	White
E	Large	Circular	Flat-Raised	Erose	Orange
F	Large	Irregular	Clumpy	Undulate	Yellow
G	Large	Irregular	Flat	Undulate	White
H	Fungal Colony				
I	Large	Circular	Raised	Entire	Tan
J	Medium	Circular	Convex	Entire	Lt. Yellow
K	Medium	Circular	Flat-Convex	Entire	White
L	Large	Circular	Flat	Entire	Cloudy White
M	Small	Circular	Pulvinate	Entire	Flesh
N	Large	Circular	Umbonate	Entire	Yellow
O	Large	Circular	Umbonate	Entire	Tan
P	X-large	Circular	Flat	Erose	Cream
Q	X-Large	Irregular	Clumpy/Stringy	Undulate	Orange
R	X-Large	Irregular	Raised	Erose	Cream
S	Medium	Irregular	Clumpy	Undulate	White
T	X-Large	Circular	Convex	Entire	Yellow
X	Medium	Filanetous	Pulvinate	Lobate	Yellow
Z	Medium	Filanetous	Flat	Filamentous	White

*Reference (29).

Columbia CNA agar allows only gram-positive organism growth, and Youssef 101 grows only yeast and fungi. With this information identifying organism species was possible. The test results were positive (growth) for Columbia CNA for all three organisms, indicating that they were gram-positive. The McConkey agar and Yousseff 101 yielded few or no organisms. With this data it was determined that organisms A, B, and D were either micrococcus or staphylococcus. Micrococcus is a common inhabitant of soil and water and is usually dispersed through air. Staphylococcus is commonly found on humans or animals. No gram-negative, yeast, or fungi were found among these three organisms.

The first set of raw data in Appendix A for the various test are from untreated exposed petri dishes. The second group of data represents dishes with Staticide® Topical Surface Solution.

The average voltage shown in the data tables is determined by adding the voltage readings taken every ten minutes from the electrostatic voltmeter and dividing this total by seven, the number of readings taken in one hour.

The relative humidity for both phases of this experiment was around 40 percent. Relative humidity did fluctuate between 30% to 60% but was usually on the low end of the scale.

Summary Comparison of Culture Totals

The data presented in Tables 2 and 3 is a summary of the Appendix A data. Totals were calculated by adding the

number of colonies found on each of the three dishes of either positive, negative, or zero charge for that particular test period. Zero charge data was considered to be the control data in these experiments. For each test period, 1 through 10, percentages are arrived at by equating the number of colony forming units (CFU) from the control (zero charge) plates to 100%. For example 37 positive CFU equal 231% relative to 16 zero CFU set at 100%. Mean ($\bar{x}$) and standard deviation (α) are calculated by leaving out the high and low values to provent extremes values from adversely influencing results. A bar graph representation of Table 2 is given in Figure 4, and for Table 2 in Figure 5. The bar graph conversion allows comparisons to be drawn between the positive and negative charged plates.

Percentage Comparison of Microorganism Types

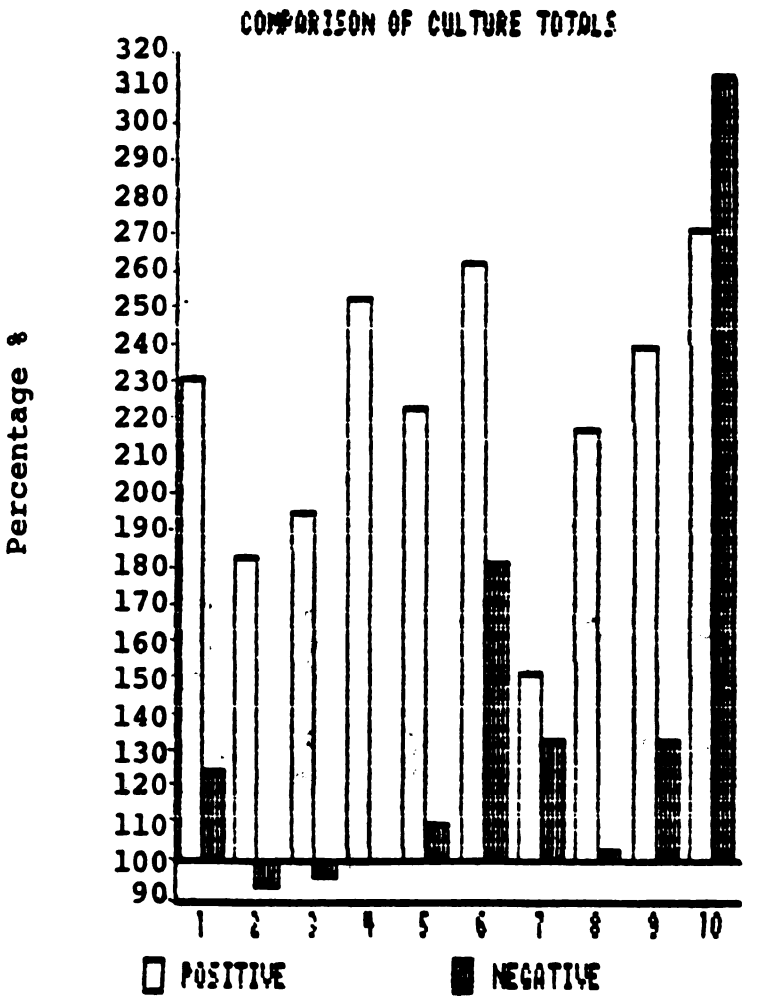
The next step was to determine whether a different charge attracts greater numbers of a particular organism. Totals for each type of organism for a given charge were calculated from data given in Appendix A. Accumulated totals for each charge and organism types for the untreated cultures are given in Table 4, and a graphic illustration is given in Figure 6. Each organism percentage is based on the total number of organisms attracted by a particular charge relative to the attraction of the control or zero charged plates (100%). In analyzing the data it is apparent that there is a far greater number of organism A than all other organisms, averaging about 75% of the total count. In order

Table 2. Culture Totals Per Test Period for Each Set of Three Charged Plates. Percentages in Parenthesis are Relative to the Zero Charge, Control Total.

	Positive Count	Zero Count	Negative Count
#1	37 (231%)	16 (100%)	20 (120%)
#2	66 (183%)	36 (100%)	34 (94%)
#3	64 (194%)	33 (100%)	32 (97%)
#4	38 (253%)	15 (100%)	15 (100%)
#5	212 (223%)	95 (100%)	104 (110%)
#6	97 (262%)	37 (100%)	65 (181%)
#7	50 (152%)	33 (100%)	44 (133%)
#8	52 (217%)	24 (100%)	28 (104%)
#9	36 (240%)	15 (100%)	20 (133%)
#10	19 (271%)	7 (100%)	22 (314%)
$\bar{x}$	55.0	26.1	33.1
σ	20.6	9.8	15.3

Table 3. Culture Totals Per Test Period for Each Set of Three Charged Plates with Staticide® Topical Solution. Percentages in Parenthesis are Relative to the Zero Charge, Control Total.

	Positive Count	Zero Count	Negative Count
#1	25 (179%)	14 (100%)	31 (221%)
#2	54 (108%)	50 (100%)	50 (100%)
#3	32 (168%)	19 (100%)	28 (147%)
#4	31 (163%)	19 (100%)	16 (84%)
#5	115 (230%)	50 (100%)	58 (116%)
#6	100 (135%)	74 (100%)	78 (105%)
#7	44 (191%)	23 (100%)	30 (130%)
#8	17 (243%)	7 (100%)	15 (214%)
#9	11 (157%)	7 (100%)	9 (129%)
#10	9 (150%)	6 (100%)	10 (167%)
$\bar{x}$	39.3	23.6	29.8
σ	28.2	17.2	16.9



Zero charge equal to 100%

Figure 4. Percentage of Positive, Negative and Zero Charge for Each Untreated Test Period.

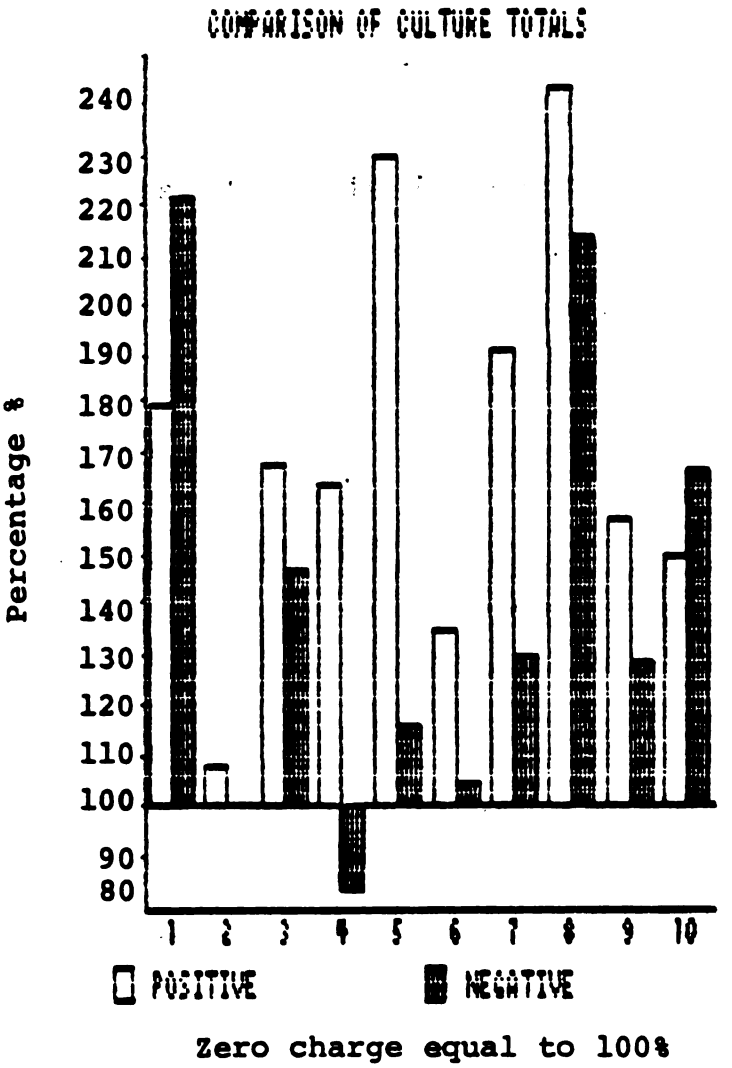


Figure 5. Percentage of Positive, Negative, and Zero Charge (With Staticide Topical Solution) for Each Test Period.

of diminishing occurrence the organisms may be ranked B, D, All Others, and C, respectively. This holds true for positive, negative, and zero charged type for both treated and untreated dishes. Data for the topical surface solutions (Staticide®) treated samples are given in Table 5 and the graphic illustration in Figure 7. These percentages were calculated using the same method as for untreated samples. The differences in organisms attracted for each charge, shown in Figure 6 and 7 indicate that each charge attracted about the same percentages of each organism type as did the untreated plates. The main difference was that the positive plates attracted a greater number of each type organism that either negative or zero charged plates. There was no shift in population type between the three types of samples (Figures 6 & 7).

ANOVA--Analysis of Variance

This is a technique whereby the total variation present in a set of data is separated into several components. Each of these components is a source of variation and this analysis allows us to determine the contribution of each sources to the total variation (8).

In analyzing the data the one-way analysis of variance was used, essentially an extension of the t-test for the differences between means (13). We classified the sample units according to one criterion, the treatment group they belong to. This allows us to test the significance of the plate charge in the attraction of airborne organisms.

Table 4. Accumulated Totals by Organism Type for Untreated Cultures for Positive, Negative, and Zero Charge.

		A	B	C	D	All Others	Total
Positive:	Total	514	76	14	30	37	671
	Percentage	76.6	11.4	2	4.5	5.5	100%
Negative:	Total	281	56	14	17	16	384
	Percentage	73.2	14.6	3.6	4.4	4.2	100%
Zero Charge:	Total	233	41	6	14	17	311
	Percentage	74.9	13.1	2	4.5	5.5	100%

Table 5. Accumulated Totals by Organism Type for Topical Solution Treated Cultures for Positive, Negative, and Zero Charge.

		A	B	C	D	All Others	Total
Positive:	Total	336	40	9	27	29	441
	Percentage	76.2	9.1	2	6.1	6.6	100%
Negative:	Total	236	39	8	17	25	325
	Percentage	72.6	12	2.5	5.2	7.7	100%
Zero Charge:	Total	211	23	12	17	16	279
	Percentage	75.6	8.3	4.3	6.1	5.7	100%

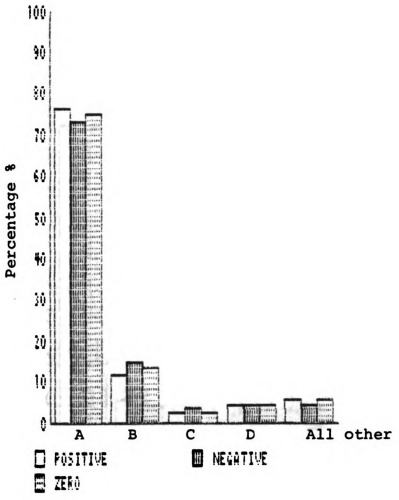


Figure 6. Percentage Comparison by Microorganism Type for Positive, Negative, and Zero Charge for Untreated Dishes.

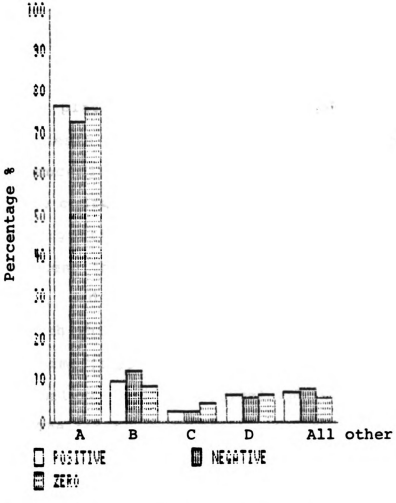


Figure 7. Percentage Comparison by Microorganism Type for Positive, Negative, and Zero Charge for Treated Dishes.

Analysis of variance is used to both estimate and test hypotheses about population variances and to estimate and test hypotheses about population means. The test hypothese in this experiment was broken down into three parts: 1) Contrast 1, the effect of positively charged plates verses that of uncharged control plates in the attraction of airborne organisms, 2) Contrast 2, positively charged plates verses negative charged plates, and 3) Contrast 3, negatively charged plates verses uncharged control plates.

For each contrast we set the null hypothesis (H_o) to be that the means are not equal, or in other words, a difference in the attraction of microorganisms does exist between the charged plates compared in the contrast. The alternative hypothesis (H_1) is that the means are equal, i.e. that no difference in the attraction of microorganisms exist between the charged plates compared in the contrasts. The level of significance (α) was set at .05. If our T probability is less than .05, we accept the null hypothesis "are not equal", and reject the alternative hypothesis, "are equal". With this we can expect to make the wrong decision 5% of the time (24).

The analyses and statistical conclusions are given in Table 6. The first comparison is for the overall experiment for untreated samples followed by an analysis for each individual test period (1 through 10). Table 7 contains the same information for the topical surface treated samples.

Table 6. Analysis of Variance for Untreated Samples.

		T Value	H_o (less than .05)
Overall Experiment:	Contrast 1	.003	Accept
	Contrast 2	.017	Accept
	Contrast 3	.303	Reject
Test Period 1:	Contrast 1	.135	Reject
	Contrast 2	.175	Reject
	Contrast 3	.252	Reject
Test Period 2:	Contrast 1	.048	Accept
	Contrast 2	.061	Reject
	Contrast 3	.863	Reject
Test Period 3:	Contrast 1	.043	Accept
	Contrast 2	.067	Reject
	Contrast 3	.942	Reject
Test Period 4:	Contrast 1	.054	Reject
	Contrast 2	.036	Accept
	Contrast 3	1.00	Reject
Test Period 5:	Contrast 1	.023	Accept
	Contrast 2	.053	Reject
	Contrast 3	.494	Reject
Test Period 6:	Contrast 1	.050	Accept
	Contrast 2	.232	Reject
	Contrast 3	.279	Reject
Test Period 7:	Contrast 1	.445	Reject
	Contrast 2	.775	Reject
	Contrast 3	.363	Reject
Test Period 8:	Contrast 1	.016	Accept
	Contrast 2	.030	Accept
	Contrast 3	.587	Reject
Test Period 9:	Contrast 1	.062	Reject
	Contrast 2	.140	Reject
	Contrast 3	.560	Reject
Test Period 10:	Contrast 1	.233	Reject
	Contrast 2	.713	Reject
	Contrast 3	.068	Reject

Table 7. Analysis of Variance for Topical Solution Treated Samples.

		T Value	H_o (less than .05)
Overall Experiment:	Contrast 1	.050	Accept
	Contrast 2	.152	Reject
	Contrast 3	.485	Reject
Test Period 1:	Contrast 1	.279	Reject
	Contrast 2	.667	Reject
	Contrast 3	.126	Reject
Test Period 2:	Contrast 1	.786	Reject
	Contrast 2	.786	Reject
	Contrast 3	1.00	Reject
Test Period 3:	Contrast 1	.074	Reject
	Contrast 2	.608	Reject
	Contrast 3	.315	Reject
Test Period 4:	Contrast 1	.106	Reject
	Contrast 2	.041	Accept
	Contrast 3	.624	Reject
Test Period 5:	Contrast 1	.040	Accept
	Contrast 2	.092	Reject
	Contrast 3	.580	Reject
Test Period 6:	Contrast 1	.103	Reject
	Contrast 2	.157	Reject
	Contrast 3	.768	Reject
Test Period 7:	Contrast 1	.327	Reject
	Contrast 2	.023	Accept
	Contrast 3	.785	Reject
Test Period 8:	Contrast 1	.175	Reject
	Contrast 2	.801	Accept
	Contrast 3	.246	Reject
Test Period 9:	Contrast 1	.124	Reject
	Contrast 2	.057	Reject
	Contrast 3	.529	Reject
Test Period 10:	Contrast 1	.288	Reject
	Contrast 2	.773	Reject
	Contrast 3	.295	Reject

CHAPTER 6

CONCLUSION

From the data obtained in this experiment, it may be concluded that there is a significant difference between the numbers of airborne microorganisms attracted to a positively charged untreated dish compared to those attracted to either negatively or zero charged dishes. In analyzing the data for the untreated samples the positively charged dish was found to attract airborne microorganisms at a significantly greater level than either negatively and zero charged dishes. There was not a significant difference between the numbers attracted by the negatively and zero charged dishes. Results varied for each individual test period, but the T values were always lower for the positive charge compared to the other charges.

In the case of the Staticide® treated samples it is more difficult to draw conclusions. The positively charged cultures were found to attract airborne microorganisms at a level significantly different than the zero charge, but not by much. When comparing positive to negative charges, and negative to zero charges on the treated samples, there was no significant difference. Unlike the untreated samples, the positively charged dishes showed little difference compared to the attraction of the negatively charged dishes.

Each individual test period for the Staticide® treated dish experiment also showed variations in acceptance or rejection in the null hypothesis, (see Table 7) probably due to the bacteriostat, Staticide®, which reduced the relative growth of airborne microorganisms on all the dishes, regardless of charge.

In both the treated and untreated phases of this experiment, there were deviations in acceptance and rejection of contrast 1, 2, and 3. This may be attributed to the open, "real life" conditions that the test was conducted in. Control over the surrounding air and air flow would result in less fluctuation. Additional experiments under controlled conditions should be run if exact counts and quantities are desired. The main finding of this project was that there are more microorganisms attracted by untreated positively charged plates than either negatively or zero charged plates. Uncontrolled environmental factors may influence the relative attractions.

It was determined during the experiment that microorganisms were transported in air as aerosols or on dust particles, and that the aerosol and dust particles are most likely negatively charged. The negatively charged particles are attracted by the positively charged petri dishes, but the charges involved are so small that attraction and or repulsion has only a limited effect. If greater charges were involved (greater than 500 volts), a larger significant difference would probably be observed.

The findings of this study may be of value to industries using plastics. Any environment which requires sterile or clean conditions where plastics are involved may require charge reduction. The food and pharmaceutical industries are using plastics in their packaging operations at an increasing rate, and the need to maintain a clean, sterile product and environment is critical. Every time plastic is brought into contact with a surface it tends to pick up a charge. This charge on the plastic material or container may be enough to attract microorganisms, eliminating product sterility.

Other applications where this problem may arise is in hospitals and to a lesser extent the home. Hospitals have many of the same sterility requirements as the food and pharmaceutical industry but in an environment containing a greater number and variety of microorganisms. Plastic soap dispensers and plastic containers may readily attract airborne organisms. This may cause a significant problem the transmission of disease. Likewise, in the home where plastic soap dispensers are used by family members, disease transmission could be facilitated by the same process.

These examples are simply illustrations of where microorganism attraction by electrostatic charge could present problems. Additional research on attraction of certain types of organisms is needed. Testing of several types of plastics other than polystyrene may produce different results. Additional research should also be conducted into the different methods of charge generation, and the

interaction of plastics with other materials to produce charge. It may be that the presence of charged plastic materials has an unsuspected influence on the spread of disease organisms.

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APPENDIX

Appendix A

TEST DATA FOR EXPOSED, UNTREATED PETRI DISHES

Lab Experiment #1	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 10/18/83	P_a: +500	6	1				7
Temp: 73°F	P_b: +500	11	1	1	1	2	16
RH: 33%	P_c: +500	12	1		1		14
							37
	O_a: 0	4	1				5
	O_b: 0	2			2		4
	O_c: 0	4	2			1	7
							16
	N_a: -500	6			1		7
	N_b: -500	4	2				6
	N_c: -500	6		1			7
							20

Lab Experiment #2	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 10/24/83	P_a: +500	15	5		1	1	22
Temp: 72°F	P_b: +500	20	2	1	1	3	27
RH: 50%	P_c: +500	11	4			2	17
							66
	O_a: 0	7	3	1			11
	O_b: 0	6	2			1	9
	O_c: 0	11	3		1		16
							36
	N_a: -500	7	2	1			10
	N_b: -500	11	2	1	1	2	17
	N_c: -500	5	1	1			7
							34

*Types are described on page 29.

Lab Experiment #3	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 10/24/83	P_a: +500	22	1		1	2	26
Temp: 72°F	P_b: +400	14	1		3		18
RH: 30%	P_c: +500	16	3			1	20
							64
	O_a: 0	13	1				14
	O_b: 0	6					6
	O_c: 0	10	2			1	13
							33
	N_a: -500	10	2				12
	N_b: -500	3	1				4
	N_c: -500	12	2		1	1	16
							32

Lab Experiment #4	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 10/25/83	P_a: +500	7	4		1		12
Temp: 72°F	P_b: +500	6	3	1			10
RH: 30%	P_c: +500	9	5	1	1		16
							38
	O_a: 0	4	1			1	6
	O_b: 0	5					5
	O_c: 0	3	1				4
							15
	N_a: -500	2	3				5
	N_b: -500	3					3
	N_c: -500	5	2				7
							15

*Types are described on page 29.

Lab Experiment #5	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 11/1/83	P_a: +500	49	1		1	2	54
Temp: 72°F	P_b: +500	75	2	1	1		78
RH: 58%	P_c: +500	70	3		1	6	80
							212
	O_a: 0	33		1	1	3	38
	O_b: 0	26				1	27
	O_c: 0	28	1			1	30
							95
	N_a: -500	24	5		1	1	31
	N_b: -500	32	2		2	2	38
	N_c: -500	30	3		1	1	35
							104

Lab Experiment #6	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 11/1/83	P_a: +500	22	1		1		24
Temp: 72°F	P_b: +500	33	1		1	4	39
RH: 58%	P_c: +500	30	2		1	1	34
							97
	O_a: 0	11	1				12
	O_b: 0	9			1		10
	O_c: 0	11			2	2	15
							37
	N_a: -500	12	1		1	1	15
	N_b: -500	11	3		1	1	16
	N_c: -500	28	3		2	1	34
							65

*Types are described on page 29.

Lab Experiment #7	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 11/7/83	P_a: +500	14	7	3	1	3	28
Temp: 72°F	P_b: +500	7	3	1			11
RH: 42%	P_c: +500	5		2	3	1	11
							50
	O_a: 0	7	6		2		15
	O_b: 0	5	3	1			9
	O_c: 0	6	2	1			9
							33
	N_a: -500	12		6		1	19
	N_b: -500	7	2				9
	N_c: -500	12	3			1	16
							44

Lab Experiment #8	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 11/7/83	P_a: +500	14	3		1	2	20
Temp: 72°F	P_b: +480	16	1			1	18
RH: 42%	P_c: +500	8	2	1	1	2	14
							52
	O_a: 0	1	2			2	5
	O_b: 0	5	2			2	9
	O_c: 0	9	1				10
							24
	N_a: -500	4		1		1	6
	N_b: -500	8	1			2	11
	N_c: -500	7	4				11
							28

*Types are described on page 29.

Lab Experiment #9	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 11/8/83	P_a: +500	4	2		2	1	9
Temp: 72°F	P_b: +500	7	3		3	2	15
RH: 38%	P_c: +500	5	4		2	1	12
							36
	O_a: 0	1	2		1		4
	O_b: 0	2	2			1	5
	O_c: 0	1	3		3		6
							15
	N_a: -500	5	3		1		9
	N_b: -450		1		1		2
	N_c: -500	5	2		1	1	9
							20

Lab Experiment #10	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 11/8/83	P_a: +400	1	1				2
Temp: 72°F	P_b: +400	5	5				10
RH: 38%	P_c: +500	2	4		1		7
							19
	O_a: 0				1		1
	O_b: 0		1				1
	O_c: 0	3	2				5
							7
	N_a: -390	5	2		1		8
	N_b: -410	1	3		2	1	7
	N_c: -390	4	2	1			7
							22

*Types are described on page 29.

TEST DATA FOR EXPOSED PETRI DISHES TREATED WITH STATICIDE® TOPICAL SOLUTION

Lab Experiment #1	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 10/18/83	SP_a: +400	7					7
Temp: 73°F	SP_b: +450	8	4			2	14
RH: 33%	SP_c: +450	4	1				5
							25
	SO_a: 0	2					2
	SO_b: 0	4	2				6
	SO_c: 0	4	1		1		6
							14
	SN_a: -500	6	2				8
	SN_b: -500	6	2				8
	SN_c: -500	10	1		1	3	15
							31

Lab Experiment #2	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 10/24/83	SP_a: +300	8	3		1		12
Temp: 72°F	SP_b: +300	16	1				17
RH: 50%	SP_c: +350	20	3		1	1	25
							54
	SO_a: 0	17	2	1	1		21
	SO_b:0	9	2	1			12
	SO_c:0	13	1		1	2	17
							50
	SN_a: -400	14	3		1	2	20
	SN_b: -300	9		3			12
	SN_c: -300	15	1			2	18
							50

*Types are described on page 29.

Lab Experiment #3	Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 10/25/83	SP_a: +500	7	1		2		10
Temp: 72°F	SP_b: +500	11					11
RH: 30%	SP_c: +500	7	1		1	2	11
							32
	SO_a: 0	6	1		1		8
	SO_b: 0	4					4
	SO_c: 0	3	2		2		7
							19
	SN_A: -500	8	2		1	1	12
	SN_B: -500	4		1			5
	SN_C: -500	8	2			1	11
							28

Lab Experiment #4	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 10/25/83	SP_a: +500	9		1	1		11
Temp: 72°F	SP_b: +500	7	1				8
RH: 30%	SP_c: +500	7	3	1		1	12
							31
	SO_a: 0	3		1			4
	SO_b: 0	7			1	1	9
	SO_c: 0	4	2				6
							19
	SN_a: -500	5	1		1		7
	SN_b: -500	5	1				6
	SN_c: -500	3					3
							16

*Types are described on page 29.

Lab Experiment #5	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 11/1/83	SP_a: +500	25		1	2	2	30
Temp: 72°F	SP_b: +500	30	1		2	2	35
RH: 58%	SP_c: +500	45	1	1	2	1	50
							115
	SO_a: 0	18		1	3	2	24
	SO_b: 0	14		1	1		16
	SOc: 0	8		1		1	10
							50
	SN_a: -500	18	1	1	1	1	22
	SN_b: -500	14	1		1	1	17
	SN_c: -500	16	1	1		1	19
							58

Lab Experiment #6	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 11/1/83	SP_a: +350	27	3		2	2	34
Temp: 72°F	SP_b: +350	33		1	2	2	38
RH: 58%	SP_c: +400	25	1	1	1		28
							100
	SO_a: 0	20		1	1	2	24
	SO_b: 0	17	1	2			20
	SO_c: 0	26	1	2		1	30
							74
	SN_a: -400	19	1		1	1	22
	SN_b: -400	25	2	1		4	32
	SN_c: -400	19	1	1	2	1	24
							78

*Types are described on page 29.

Lab Experiment #7	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 11/7/83	SP_a: +500	5	1	2	2	4	14
Temp: 72°F	SP_b: +500	5	2		3	5	15
RH: 42%	SP_c: +500	7	2		3	3	15
							44
	SO_a: 0	4		1	1	1	7
	SO_b: 0	11	2		1	3	17
	SO_c: 0	3	1	1	2	2	9
							23
	SN_a: -500	6	2		2	2	12
	SN_b: -500	5	2		2		9
	SN_c: -500	3	3		2	1	9
							30

Lab Experiment #8	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 11/7/83	SP_a: +500	2	1				3
Temp: 72°F	SP_b: +500	5					5
RH: 42%	SP_c: +500	5	3			1	9
							17
	SO_a: 0	3					3
	SO_b: 0		1				1
	SO_c: 0	2	1				3
							7
	SN_a:		1			1	2
	SN_b:	2	2			1	5
	SN_c:	4	2		2		8
							15

*Types are described on page 29.

Lab Experiment #9	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 11/8/83	SP_a: +500	1	2			1	4
Temp: 72°F	SP_b: +500	3	1				4
RH: 38%	SP_c: +500	3	2				5
							11
	SO_a: 0	1					1
	SO_b: 0	2	2				4
	SO_c: 0	2					2
							7
	SN_a: -500	1	1			1	3
	SN_b: -500	2	1				3
	SN_c: -500	3					3
							9

Lab Experiment #10	"Average" Plate Voltage	Number of Organisms by Type* A	B	C	D	All Others	Total
Date: 11/8/83	SP_a: +500	2			1		3
Temp: 72°F	SP_b: +500		2				2
RH: 38%	SP_c: +500	3				1	4
							9
	SO_a: 0	1					1
	SO_b: 0	2				1	3
	SO_c: 0	1	1				2
							6
	SN_a: -500		2			1	3
	SN_b: -500	2					2
	SN_c: -500	4	1				5
							10

*Types are described on page 29.