REVISED BRIEF OF TOXICITY DATA

The following materials summarize test data relating to the toxicology of AEM 5700 Antimicrobial, AEM 5772 Antimicrobial, and ÆGIS Antimicrobial [3-(trimethoxysilyl) propyloctadecyldimethyl ammonium chloride] and were prepared by the Toxicology Department of Dow Corning Corporation Health and Environmental Sciences Group.

The antimicrobial material is produced as a concentrate (42% actives in methanol). For use, the antimicrobial material is typically diluted to 1- 3% by weight in water. It is also available in a 72% actives concentrate. Many of the data were generated using the polymer form of this active ingredient.

REVISED BRIEF OF TOXICITY DATA PERTAINING TO AEM 5700 ANTIMICROBIAL

ORAL TOXICOLOGY

Acute Oral Toxicity

Undiluted AEM Antimicrobial has a low acute oral toxicity. There should be no problem from ingestion incidental to industrial handling. If large quantities are ingested accidentally or willfully, some injury may result; the likelihood of serious injury is remote.

Albino Rats

AEM 5700 Antimicrobial has an extremely low acute oral toxicity (LD_{50} = 12.27 gm/kg body weight) and therefore should pose no hazard from ingestion incidental to industrial handling.

SKIN CONTACT

Skin Contact - Irritation

The undiluted test material has a slight effect on intact or abraded skin. A single exposure for several hours may cause a slight irritation. Repeated and/or prolonged contact over a period of several days may cause blistering and a superficial burn. Good care and cleanliness should be exercised. Clothing or shoes grossly contaminated should be removed and cleaned before reuse.

Skin Contact - Absorption

Based on the results of the skin irritation study, AEM 5700 Antimicrobial does not appear to be absorbed through the skin in acutely toxic amounts.

Acute Dermal Toxicity - Albino Rabbits

AEM 5700 Antimicrobial has an extremely low acute dermal toxicity (LD_{50} greater than 7.95 gm/kg body weight) and therefore does not appear to present a problem from skin absorption under ordinary industrial handling conditions.

Skin Contact - Irritation

Both aqueous dilutions of the test material have essentially no effect upon intact or abraded skin. At most, this may produce a slight scaling after repeated, prolonged contact. Reasonable care and cleanliness should avert any significant response.

Human Repeated Insult Patch Test (HRIPT) with AEM 5700 Antimicrobial

A repeated insult patch test with humans was conducted with AEM 5700 Antimicrobial. The test material was evaluated as a 2.0% (w/v) aqueous solution which was prepared fresh daily.

The results of the study showed the overall incidence of skin irritating reactions to be 2/450 or 0.5%. Two subjects each reacted once to the applications of the test material. One of the reactions was classified as very slight erythema with very slight edema.

There was no evidence of skin sensitization noted with any of the subjects.

Percutaneous Absorption of AEM 5700 Antimicrobial in Rabbits

The percutaneous absorption potential of AEM 5700 Antimicrobial, 3-(trimethoxysilyl) propyldimethyloctadecyl ammonium chloride was studied in rabbits. Radiolabeled C^{14} AEM 5700 Antimicrobial (^{14} AEM 5700) was either applied to the intact clipped back of animals for 10 days or administered by a single intravenous dose. Urine and feces were collected at 24-hour intervals and tissue concentration of radioactivity was determined at the end of the ten day study period. Elimination of ^{14} AEM-5700 after parenteral administration was slow and occurred by both urine and feces (13.5% in urine; 20% in feces). No radioactivity was determined in the urine of animals treated dermally; however, 1% of the applied dose was detected in the feces of a single rabbit on days 7, 8, and 9 of the study. Tissue concentrations of ^{14}C were highest in the liver, lung and kidneys after IV administration. None was detected in tissues of animals treated dermally. The results showed that the absorption of AEM 5700 Antimicrobial through the skin of the rabbit was essentially zero. The potential hazard of the use of this antimicrobial in contact with the skin is, therefore, considered to be insignificant.

28-Day Subchronic Dermal Toxicity Study
with Fabrics Treated with AEM 5700 Antimicrobial in Albino Rabbits

This study employed a fabric (nonwoven polyester) treated with AEM 5700 Antimicrobial at 0.5%, w/w, 5% w/w, and no treatment (used as a control).

Approximately 60 square inches of fabric were allowed to contact the shaved back and abdomen of the rabbits for 6 hours per day, 5 days per week for 4 weeks (20 applications). The skin of each rabbit was pre-moistened with normal saline to simulate perspiration. After the 6-hour contact period, the fabrics were removed, rinsed with tap water and patted dry.

The results of this study indicate that no significant untoward alterations were noted with regard to mortality, reactions, effects on body weight, hematologic and clinical blood chemistry studies, urine analysis or gross and microscopic pathologic examinations among any of the test animals.

Three -Week Vaginal Irritation Study
with Panty Hose Fabric Treated with AEM 5700 Antimicrobial in Beagle Dogs

A 3-week vaginal irritation study with treated panty hose fabrics* (cotton and nylon) was conducted using sexually mature purebred beagle dogs. Each test animal (four with treated cotton fabric, four with treated nylon fabric) received 15 intravaginal applications, each of which allowed a 24-hour exposure period. Each treated control animal (four with untreated cotton fabric, four with untreated nylon fabric) received the same type of application procedure. Two untreated control animals were utilized during the study for purposes of comparison.

Vaginal examinations performed during the course of the study and gross and histologic examinations conducted at its termination revealed no significant differences between test and treated control animals exposed to either the cotton or nylon fabrics.

* *Application was by conventional technique and actual deposition of the antimicrobial was in the range of 0.2% based upon garment weight.*

32-Day Human Wear Test with Socks Treated with AEM 5700 Antimicrobial

Various sock materials (wool, cotton, nylon, ORLON® and SPANDEX®) were treated with AEM 5700 Antimicrobial at 0.35% owf (on weight of fiber) for safety evaluations of skin irritation and skin sensitization properties. The antimicrobial (AEM 5700) treated socks were evaluated on 23 male subjects for a total of 32 continuous days.

The results of the investigation showed the antimicrobial treated socks to be free from any observable skin irritation or skin sensitization.

Three-Month Wear Test for Treated Athletic Socks with AEM 5700 Antimicrobial

Various sock materials (wool, cotton, nylon, ORLON and SPANDEX) were treated with AEM 5700 Antimicrobial at 0.35% owf (on weight of fiber) for safety evaluation of potential hazard to skin under normal wear conditions. The antimicrobial (AEM 5700) treated socks were evaluated for skin irritating and skin sensitizing properties on 44 young male subjects for an entire football season (approximately 90 continuous days).

The results of the investigation showed the male subjects to be free from any observable skin irritation or skin sensitization at the end of the test period.

Inhalation

Acute Vapor Inhalation Toxicity Study with AEM 5700 Antimicrobial in Albino Rats

The vapors were generated by bubbling air through the undiluted AEM 5700 Antimicrobial and passing the resulting vapors directly into an exposed chamber containing ten rats. The nominal concentration was 81.9 mg/liter and the exposure time was 4 hours. No mortalities occurred during the exposure or within 14 days after the exposure. Body weights appeared normal and no gross pathologic alterations were noted in any rats at the end of the 14-day observation period. Ataxia was observed in the test animals during the exposure.

The acute four-hour vapor inhalation LC_{50} for AEM 5700 Antimicrobial is greater than 81.9 mg/liter.

Genetic

Host-Mediated Assay for Detection of Mutations Induced by AEM 5700 Antimicrobial

Mutations can arise spontaneously or can be induced. Systems involving bacteria, insects, or molds have been devised to investigate compounds that may induce genetic changes. However, the relevancy of these tests, as related to mammals, is difficult to measure. The host-mediated assay is a method of screening potential mutagens in which both a bacterial system and a mammalian system are used. Male albino rats are treated with a test compound. After a period of treatment, during which time the compound can be metabolized, the host animal is inoculated with bacteria

in which reverse mutations can be measured. Following exposure to the compound and/or its metabolites *in vivo*, the bacteria are recovered and the number of revertants (mutants) is determined.

Host-mediated mutagenic assays using albino rats treated with AEM 5700 Antimicrobial were conducted. Animals were treated for 5 consecutive days at dose levels of either 100 or 1,000 mg/kg AEM 5700 Antimicrobial. The animals were then inoculated with a histidine dependent strain of *S. typhimurium* (strain G-46). After a 3-hour exposure, the bacteria were recovered and the number of bacteria no longer dependent on an external source of histidine was determined. The positive control group rats each received a single intramuscular (i.m.) injection of 100 mg/kg of DMN (dimethylnitrosamine) prior to inoculation with bacteria.

No deaths or untoward behavioral reactions occurred during the investigation.

The number of revertants (mutants) obtained from rats treated with AEM 5700 Antimicrobial revealed no differences from the number of revertants in the control animals. Strain sensitivity to a known mutagen was demonstrated in albino rats.

- *The material tested was prepared by placing AEM 5700 in water (hydrolysis) and recovering the resulting solid (hydrolyzate). This material should be equivalent to the treatment of a surface from an aqueous bath.*

Mutagenicity Evaluation of AEM 5700 Antimicrobial

The objective of this study was to evaluate the test materials for genetic activity in a microbial assay (Ames et al., Mutation Research 31:347, 1975) with and without the addition of mammalian metabolic activation preparations.

TEST MATERIALS

A.	AEM 5700 (Lot No. BN126008)	43% 3-(trimethoxysilyl)- propyldimethyloctadecyl ammonium chloride.
	50% Methanol	
B.	AEM 5700 X9-5706 (Ref. E-2054-149)	Prepared hydrolyzate of AEM 5700
C.	Artificial sweat extract	AEM 5700 treated Gold Cup® style 7953 (75% orlon/25% nylon); Burlington socks. Extracted with artificial sweat (Blood and other Body Fluids; P.L. Altman and D.S. Dittmar, FASEB, 1961) for 24 hours on a New Brunswick rotary shaker at 125 rpm.
D.	Artificial sweat extract	Sock control. Same as (C) above without AEM 5700 treatment.

None of the materials tested were bacterial mutagens, with or without metabolic activation in the Ames Bacterial Assay system.

Activity of AEM 5700 Antimicrobial in *in vitro* mammalian cell transformation assay in the absence of exogenous metabolic activation.

The objective of this study was to employ the BALB/3T3 Clone A31 mouse cell line in the absence of exogenous metabolic activity in order to investigate the *in vitro* morphological transforming potential of AEM 5700 Antimicrobial, 3-(trimethoxysily) propyldimethyloctadecyl ammonium chloride.

The test compound gave no statistically significant (P 0.05) evidence of morphological transformation relative to the negative control condition. However, it should be noted that the agent did induce one morphological transformed

(Type III) focus at each of two different delivered doses, i.e., 1:6 x 10^4 dilutions. To confirm the reproducibility of this observation, the activity of AEM 5700 Antimicrobial was evaluated in an *in vitro* mammalian cell transformation assay in the presence of exogenous metabolic activity.

Activity of AEM 5700 Antimicrobial in *in vitro* mammalian cell transformation assay in the presence of exogenous metabolic activation

The objective of this study was to employ the BALB/3T3 Clone A31 mouse cell line in the presence of exogenous metabolic activity in order to investigate the in vitro morphological transforming potential of AEM 5700 Antimicrobial 3-(trimethoxysilyl) propyldimethyloctadecyl ammonium chloride.

Under the conditions of this test, AEM 5700 Antimicrobial was ineffective in producing Type III foci at all of the concentrations tested. Treatment with Benzo (a) pyrene (BP, the positive control chemical) in the presence of exogenous S-9, induced the formation of three morphologically transformed foci (transformation frequency of 1.61 x 10^{-4}), whereas the negative control condition (S-9 + 0.25% DMSO) failed to induce spontaneous transformants.

Reproductive

Teratogenic Study with AEM 5700 Antimicrobial in Rats

A teratogenic study was conducted employing albino rats treated orally with AEM 5700 Antimicrobial at dose levels of either 0, 100, or 300, or 1000 mg/kg of body weight during gestation days 6 through 15. The following results were obtained during the investigation.

Maternal body weights and body weight gains of dams exposed to AEM 5700 Antimicrobial were similar to those of the concurrent group of control dams. There were two deaths among the dams during the investigation (1 nongravid 1000 mg/kg dam and 1 pregnant 100 mg/kg dam). Necropsy examination of these dams failed to reveal any correlation with the exposure to AEM 5700 Antimicrobial. Dams exposed to the test material displayed no untoward behavioral reactions. Results of the sacrificed dams on gestation day 20 revealed no reproductive effects which could be correlated with the exposure to the tested material.

Body weight of fetuses obtained from dams given AEM 5700 Antimicrobial were essentially the same as those of concurrent control fetuses. The examination of fetuses for external abnormalities revealed no major structural anomalies among the fetuses obtained from dams given AEM 5700 Antimicrobial which could be attributed to the maternal exposure to the test material. Similar findings were observed among the test and control fetuses during the examination of the fetal skeletal and internal development.

AEM 5700 Antimicrobial was found to be non-teratogenic in this test system.

- The material tested was prepared by placing AEM 5700 Antimicrobial in water (hydrolysis) and recovering the resulting solid (hydrolyzate). This material should be equivalent to the treatment of a surface from an aqueous bath.

Evaluation of Effects on Elevated Levels of Normal Skin Bacterial Flora
with Fabrics Treated with 3-(Trimethoxysilyl) Propyldimethyloctadecyl Ammonium Chloride

By W. Curtis White, ÆGIS Environments, Midland, MI
Benny L. Triplett, Burlington Industries, Greensboro, NC

Summary

Concern has been expressed that antimicrobial agents used on fabrics can affect normal skin bacterial flora and give rise to adapted or dominant species. This imbalance could have negative effects. This work was undertaken to: (A) evaluate the effects of fabrics treated with 3-(trimethoxysily) propyldimethyloctadecyl ammonium chloride (ÆGIS Antimicrobial Treatment) on elevated populations of normal skin bacteria under an occlusive dressing and (B) evaluate the retrievable counts of the target bacteria associated with treated and nontreated fabrics.

Results show clearly that fabric treated with 3-(trimethoxysily) propyldimethyloctadecyl ammonium chloride exerted no untoward effects on the elevated skin target bacterial populations under the occlusive dressing when compared to the untreated fabric. Testing of the target bacterial flora in the fabric itself showed 99.96 to >99.99 percent reduction in the treated fabric as compared to the untreated fabric.

Key words: Antimicrobial, Skin, Textiles, Silane

Introduction

Treatment of fabrics with antimicrobial agents (AA) to inhibit bacteria and fungi associated with body/fabric odor has been commercially practiced for many years [2,9]. Concern that these agents could affect normal skin bacterial flora and give rise to adapted or dominant species and negative effects of these imbalances has been expressed.

The purpose of this work was to evaluate the effects of fabrics treated with 3-(trimethoxysily) propyldimethyloctadecyl ammonium chloride (SiQAC) ÆGIS Antimicrobial Treatment) on elevated populations of normal skin bacteria under an occlusive dressing and the survival of bacteria in the treated fabric.[1]

Skin colonization by bacteria is a normal and healthy process. The number and types of organisms can vary greatly and shifts from healthy to unhealthy conditions can occur quite readily. Skin microflora are associated with odors and disease. The role of the skin as a transfer (person-to-person), object-to-object, or person-to-object) agent of microorganisms is also noted.

ÆGIS Antimicrobial Treatment for this report is synonymous with BIOGUARD® Fabrics and with AEM 5700 Antimicrobial Agent treated fabrics.

The skin is clearly the definer of its own flora. Variable factors such as secretory antibodies, lipidlipopreotein availability, desquamation, rupture or abrasion, humidity, pH, bacterial adherence, microbial potentiation or antagonism and external toxicants influence the nature of the bacterial flora.

Insights relating to the relations of AA to skin microflora can be gained from the voluminous literature on AA used in soaps. Marzulli and Bruch[5] described the following risks associated with the contact of skin by AA soaps: 1) skin irritation and/or sensitization, 2) photosensitizations, 3) selective antimicrobial activity, 4) percutaneous absorption, 5) cross-sensitizations, 6) ability to be formulated and 7) photo allergenic responses. We would add the potential of AA to stimulate mutational or inductive adaptation to this list of risks. Depending on the durable nature of AA used on fabrics, some or all of the above risks could apply.

The durable nature, broad spectrum antimicrobial activity, and positive results of in-use odor suppression by sock textile fabrics treated with 3-(trimethoxysily) propyldimethyloctadecyl ammonium chloride has been reported by Gettings and Triplett.[2] Their work included a demonstration of activity against a variety of laboratory and skin isolate bacterial strains (Table I).

An AA activity on skin can be evaluated by quantitating its ability to prevent the increase in the population of normal skin flora which under controlled conditions occurs beneath an occlusive dressing. The simple maneuver of applying impermeable plastic film (SARAN® WRAP) reinforced with adhesive tape over areas of the forearm allows for the increased temperature and moisture that allows the normal skin flora to propagate from a level of hundreds of organisms/cm^2 to over a million/cm^2 in a 48 hour period. An effective AA will prevent such an increase.[4] This technique gives reproducible data and has the advantage of each individual serving as their own control when a proximal area is occluded without the test agent.

MATERIALS AND METHODS

Fabric Preparation

AEM 5700 Antimicrobial Agent (42% SiQAC in methanol) was used to treat 60/40 orlon/nylon sock fabric at 1% as is in a commercial sized paddle machine at a 30:1 liquor to goods ratio at 110°F allowing twenty minutes for dynamic exhaustion. Untreated control socks were exposed to the same conditions without the presence of the AA. After treatment, the socks were extracted in a centrifugal extractor and dried for twenty minutes at approximately 230°F in a forced-air tumble drier. The final step was a boarding process where the socks are mounted on a board (flat metal "foot and angle") and subjected to 280°F steam for approximately eighteen seconds. This treatment is representative of industrial processes except that the 1% treatment level would generally be considered high. High, both from its surface reactive properties as well as from its ability to effect microbial reduction and the benefits of that reduction.

Effects on Skin Bacterial Flora

Ten healthy volunteers, none using soap containing antimicrobial substances, were studied as follows: Three areas of 25cm^2 each were delineated on each forearm. On one arm, one site was covered with only SARAN® WRAP and then adhesive tape and sites two and three were covered with a single 25cm^2 swatch each of untreated sock fabric and then covered with SARAN® WRAP and adhesive tape. On the other arm, sites were prepared as above except that the 25cm^2 swatches were treated with the AA SiQAC. After 48 hours of occlusion, quantitative bacteriological cultures were obtained using the following retrieval technique.

Retrieval Technique

The localized detergent scrub technique of Williamson and Kligman was used.[13] In this method, a 3.8cm^2 area of skin is outlined by a sterile glass cup into which one ml. of a fresh solution of 0.1% Triton® X-100 surfactant was added and stirred vigorously for one minute. This procedure was repeated with a second one ml. of Triton® and the two samples were pooled. Ten fold serial

dilutions were made in 0.05% phosphate buffered Triton® and standard microbiological plate counts were made as below.

Media

Standard microbiological plate counts were made with Trypticase Soy Agar (T.S.A. – a general growth media), T.S.A. with Tween-80 and lecithin added as neutralizers and to enhance growth of lpophilic diptheroids and MacConkey's agar for tentative enumeration of gram (-) organisms. Plates were incubated at 35°C for 48 hours and then at room temperature for an additional 48 hours before counting using standard counting techniques.

Effect on Fabric Bacterial Flora

In a separate experiment, five healthy volunteers were selected as above. Sample mounting was done as above except that a treated fabric sample, an untreated fabric sample and a site with no fabric were used on each arm.

Retrieval Technique

The fabric samples were cut into four equal parts (6.25cm^2) using sterile techniques. Each sample was shaken on a Burrell Wrist Action Shaker (10 setting) for three minutes in 100 ml. of phosphate buffer modified with 0.1% of Triton® X-100. Ten fold serial dilutions were made in a 0.05% phosphate buffered Triton® and standard microbiological plate counts were made as above. Baseline studies using test fabric seeded by padding with various levels of Klebsiella pneumoniae ATCC 4352 and Staphylococcus aureus ATTCC 6538 showed that retrievals of at least 99% of the seeded bacteria could be expected after three minutes of agitation as above.

RESULTS

Effects on Skin Bacterial Flora

The results are tabulated in Table II. Good occlusion was obtained and the SARAN® WRAP control sites all supported a good growth, 6.0 X 10^5 to 9.2 X 10^6/cm^2. No significant differences in species types were noted between the control site, the fabric control site or the treated fabric site. Therefore, for the purposes of this paper, we will deal only with total populations. Both the untreated sock fabrics and the sock fabrics treated with SiQAC allowed a growth of bacteria less than the control sites although colonial morphologies were essentially similar. On each individual, the bacterial counts on the control sock fabric sites and the treated sock fabric sites were roughly within the same logarithm as shown in Table III. Since bacterial flora of skin are considered log-normally distributed, statistical analysis must be done after log transformation. When this is done, no statistical difference can be seen between the sites tested with control sock fabrics and those tested with treated sock fabrics ($p=0.6$). Comparison of untreated sock fabric sites with control sites shows that the untreated sock fabric significantly suppressed the expansion of the target skin bacterial flora under SARAN® WRAP ($p=0.001$). Likewise, the treated sock fabrics significantly suppressed the target bacterial population ($p=0.001$).

Effects on Fabric Bacterial Flora

Data generated during this work are tabulated in Table IV. As in the previous experiment, good occlusion was obtained and the SARAN® WRAP control sites all supported a good growth, 5.8 X 10^5 to 4.6 X 10^6/cm^2. Variation between right and left arm samples was not significant. As previously, the bacterial species identification was not significantly variable so total counts are used in the calculations. Comparing the skin bacterial flora control with the retrievals from the untreated control

fabric one can note the large number of organisms associated with the test fabric. Making a similar comparison with the SiQAC treated fabric, one notes that very few organisms were retrieved. Percent reductions comparing retrieval numbers from the control fabric and the treated fabric can be calculated by the formula:

$$\% \text{ Reductions} = \frac{\text{Control Fabric Count-Treated Fabric Count}}{\text{Control Fabric Count}} \times 100$$

Percent reductions ranged from 99.96 to > 99.99% for the SiQAC Treated fabric.

Discussion

The commercial utility of treating a fabric with an antimicrobial agent so that the negative effects of microorganisms such as odor, deterioration, defacement and the presence of medically significant microorganisms can be minimized has been practiced for many years. The ability to accomplish this without causing change of the normal skin bacterial flora should be of considerable value. Diffusing types of AA that can leave the fabric and contact the skin present an opportunity for microorganisms to adapt through mutational or inductive biochemical processes.[11] The practical effects of such a phenomenon have not been fully invested. Diffusing AA may also cause skin irritation and sensitization responses as well as have the potential for percutaneous absorption.

A unique technology, the AEGIS Antimicrobial Treatment based on 3-(trimethoxysilyl) propyldimethyloctadecyl ammonium chloride, that chemically reacts with fabrics and acts as an immobilized antimicrobial has been thoroughly described.[3,6,8,10] This treatment offers the unique advantages of minimizing the chance for toxicological interaction when in contact with the skin. Skin irritation and sensitization tests show that no untoward effects are observed from the neat chemical or from the fabrics treated with the chemical.[1] Additionally, a thorough percutaneous absorption study of water solutions of the neat chemical (its most mobile chemical form) showed no untoward effects.[7] Data have also been presented that show the unlikelihood that adaptive processes are initiated in the presence of the AEGIS Treatment.[12]

The test fabrics in this study showed the ability to reduce elevated populations of natural skin flora (in vivo) when the organisms were associated with the fabrics yet they did not affect the organisms on the skin. These observations are consistent with the reported chemical and antimicrobial nature of SIQAC.[3,6,8] Data presented in the part of this work entitled, Effects on Fabric Bacterial Flora, show reductions of organisms consistent with self-sanitizing surface claims.

Both types of sock fabrics did alter the environment in the occluded system such that skin retrievals from the skin were suppressed when compared to the normal increase in population that occurs under SARAN® WRAP occluded skin. Data from the retrievals made from the socks support this variation and show that the fabric becomes the favored substrate for the bacterial population increase. This is probably accounted for based on the predicted rise in humidity of the fabric as compared to the skin.

Interpretation of the data allows us to conclude that the test sock fabrics treated with SiQAC (AEGIS Antimicrobial Treatment) do not exert an antibacterial action on skin flora greater than that of similar socks not treated. We can also conclude from the data that the treated fabrics act as a self-sanitizing surface showing at least 99.9% reduction of the target bacteria.

TABLE I

EFFECTIVENESS OF AEGIS ANTIMICROBIAL TREATMENT SOCKS AGAINST A VARIET OF LABORATORY AND SOCK ISOLATE ORGANISMS[1]

BACTERIA	% BACTERIAL REDUCTION[2]
Micrococcus sp. I	99
Staphylococcus epidermidis	96
Enterobacter agglomerans	90
Acinetobacter calcoaceticus	99
Micrococcus sp. II	100
Micrococcus sp. III	99
Staphylococcus aureus (pigmented)	99
Staphylococcus aureus (nonpigmented)	99

[1] Adapted from Gettings, R.L. and B.L. Triplett$_2$

[2] Percent reduction as measured against an untreated control sock using AATCC-100 Test Protocol (Padding Test)

TABLE II

TOTAL COUNTS BACTERIAL DENSITY/CM^2
48 Hours Occlusive

Left Arm				Right Arm		
Subject	**SARAN® Control**	**CS**	**CS**	**AS**	**AS**	**AS**
1	$6.0x10^5$	$4.0x10^4$	$4.4x10^4$	$1.8x10^5$	$2.8x10^4$	$4.4x10^4$
2	$3.0x10^6$	$1.0x10^6$	$1.6x10^6$	$2.0x10^6$	$1.4x10^6$	$2.1x10^6$
3	$9.2x10^6$	$4.7x10^4$	$6.2x10^4$	$1.0x10^5$	$1.5x10^6$	$1.2x10^6$
4	$8.0x10^5$	$1.4x10^3$	$5.2x10^3$	$2.4x10^3$	$9.2x10^3$	$2.3x10^4$
5	$9.0x10^5$	$2.8x10^5$	$3.2x10^5$	$7.2x10^4$	$1.0x10^5$	$2.4x10^4$
6	$1.2x10^6$	$2.6x10^5$	$3.2x10^5$	$4.4x10^5$	$1.0x10^5$	$2.4x10^5$
7	$8.0x10^5$	$1.0x10^5$	$2.2x10^5$	$1.0x10^4$	$7.0x10^5$	$2.0x10^5$
8	$6.0x10^5$	$4.0x10^5$	$1.0x10^4$	$4.2x10^3$	$1.1x10^4$	$3.0x10^4$
9	$2.2x10^6$	$5.6x10^4$	$7.2x10^4$	$1.1x10^5$	$7.0x10^5$	$2.0x10^5$
10	$1.1x10^6$	$3.2x10^4$	$1.0x10^4$	$1.0x10^4$	$2.2x10^4$	$1.0x10^5$

CS = Control Sock; no antibacterial substance

AS = AEGIS Treatment

TABLE III

LOG TRANSFORMATIONS
BACTERIAL DENSITY LOG/cm²
48 Hours Occlusive

Subject	SARAN® Control	Control Sock	SYLGARD® Treatment
1	5.3981	4.2512 4.2754	5.1514 4.1905 4.2754
2	6.1995	6.0000 6.1445	6.1585 6.1380 6.1622
3	6.8318	4.2951 4.4169	5.0000 6.1413 6.1318
4	5.6310	3.1390 3.3311	3.1738 3.8414 4.1698
5	5.7943	5.1905 5.2089	4.5248 5.1271 4.1738
6	6.1318	5.1820 5.2089	5.2754 5.0000 5.1738
7	5.6310	6.0000 5.1679	4.1271 5.2089 3.0000
8	5.3981	4.2512 4.0000	3.2680 4.1288 4.1995
9	6.1660	4.3631 4.2089	5.1288 5.5012 5.1585
10	6.0000	4.5258 4.0000	4.1585 4.1660 5.0000

TABLE IV

TOTAL COUNT BACTERIAOLOGICAL LEVELS AS RETRIEVED FROM 48-HOUR OCCLUDED SKIN MOUNTED FABRICS

Test Subject	SARAN® Control[1] (skin retrieval) organisms/cm^2	Untreated[2] Control Fabric (fabric retrieval)[3] organisms/cm^2	SiQAC Treated[2] Fabric (fabric retrieval)[3] organsisms/cm^2
1) Right Arm	$2.1x10^6$	$1.6x10^6$	380
1) Left Arm	$1.8x10^6$	$1.2x10^6$	100[4]
2) Right Arm	$6.8x10^5$	$6.1x10^5$	100
2) Left Arm	$8.1x10^5$	$7.0x10^5$	100
3) Right Arm	$4.6x10^6$	$3.7x10^5$	680
3) Left Arm	3.8x106	$2.4x10^6$	720
4) Right Arm	$8.4x10^5$	$7.3x10^5$	100
4) Left Arm	$5.8x10^5$	$4.1x10^5$	100
5) Right Arm	$1.5x10^6$	$9.2x10^5$	210
5) Left Arm	$1.8x10^6$	$1.2x10^6$	480

1 Method of Williamson and Kligman[13]
2 Shaker Retrieved
3 Average of three pour plates
4 Sensitivity of the retrieval technique

SYLGARD® Antimicrobial Treatment is a registered trademark of Dow Corning Corporation.
BIOGUARD® textiles is a registered trademark of Burlington Industries.
SARAN® Wrap brand plastics film is a registered trademark of the Dow Chemical Company.
TRITON® Surfactant is a registered trademark of Rohm and Haas Corporation.

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PERCUTANEOUS ABSORPTION OF AN ANTIMICROBIAL ORGANOSILICON QUARTERNARY AMMONIUM CHLORIDE* IN RABBITS

Waheed H. Siddiqui, James R. Malek, E. Stanton and E.J. Hobbs
Toxicology Laboratory, Dow Corning Corporation, Midland, MI 48640, USA

Key words: antimicrobial agent; quaternary silsesquioxanes; percutaneous absorption; organosilicon quaternary ammonium chloride; surface active material; dermal penetration or dermal penetration or dermal absorption; radioactive tracers; carbon-14 tracers.

The percutaneous absorption of an organosilicon quaternary ammonium chloride (^{14}C)-3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (^{14}C-Si-QAC) was studied in rabbits. Aqueous solution of ^{14}C-Si-QAC was either applied to the intact, clipped back of animals for 10 days or administered by a single intravenous dose. Urine and feces were collected at 24 hr intervals and tissue concentration of radioactivity was determined at the end of the 10-day study period. Elimination of ^{14}C-Si-QAC after parenteral administration was slow and occurred by both urine and feces (13.5% in urine and 20% in feces). No radioactivity was found in the urine of dermally treated animals. Tissue concentrations of ^{14}C were highest in the liver, lung and kidneys after i.v. administration. None was detected in tissues of dermally treated animals. The results showed that the absorption of ^{14}C-Si-QAC through the skin of the rabbit was essentially zero. The potential absorption hazard of the use of this antimicrobial agent in contact with the skin is therefore considered to be insignificant.

INTRODUCTION

An organosilicon quaternary compound, 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (Si-QAC) that chemically binds to a variety of substrates, is a broad spectrum antimicrobial agent. [1,2] This compound is used to provide an antimicrobial treatment for many types of fiber, fabric and thread that is effective against both Gram-positive and Gram-negative bacteria, as well as various fungi and yeasts. Since this is a non-leachable treatment with a very low order of toxicity, Si-QAC is being substituted for certain leachable antimicrobials. The intimate long-term contact of Si-QAC treated fabric with the skin indicated the need to evaluate its dermal absorption potential. This paper reports the results of a percutaneous absorption study following the dermal application of ^{14}C labeled Si-QAC (^{14}C-Si-QAC) on rabbits.

EXPERIMENTAL

The method used for measuring percutaneous penetration of ^{14}C-Si-QAC was the procedure of Feldmann and Maibach,[3,4] where absorption is quantified on the basis of the percent of radioactivity excreted in urine for 10 days following application of a known amount of the labeled compound to the skin. To correct for excretion of radioactivity by other routes and retention of radioactivity in the body, urinary excretion data obtained after dermal application of the compound are adjusted in accordance with the urinary excretion after

intravenous dosage. This method has been adopted to study the percutaneous absorption in monkeys and humans.[5,6]

Chemicals

The test material, 3-(trimethoxysilyl)-propyldimethyl (1-^{14}C) octadecyl ammonium chloride (Sp. Act. =7.98 mCi g^{-1}; Lot No. 1202-244; Assay No. 80-197033) was custom synthesized by New England Nuclear (Boston, MS) on Dow Corning=s directions. The purity of this radioactive compound was greater than 98.8%. This carbon-14- labeled compound (^{14}C-Si-QAC) represents the active ingredient of Dow Corning7 5700 Antimicrobial Agent. Protosol7, Aquasol7 and Econofluor7 were also purchased from New England Nuclear.

Animals and treatment

The animals, New Zealand White rabbits (2.5-3.0kg), were obtained from Langshaw Farms, Augusta, Michigan. All animals used were thoroughly screened for health status and kept under observation for 10 days prior to experimental use, during which time they were checked for general physical health and suitability as test animals. Animals which did not manifest clinical signs of disease were assigned to the study. The animals were housed in rooms maintained at 20-24□C and 40-50% relative humidity. At the initiation of the study, all the rabbits were randomly divided into two groups of six animals (three of each sex) and each rabbit was then assigned a number. These groups of rabbits were administered the test material either by dermal or intravenous routes.

Twenty-four hours prior to the dermal application, the backs (between the levels of fore limbs axillae and lower hip) of six rabbits were clipped free of hair with electric animal clippers. A rectangular area (16 cm^2) was marked on the skin and 100□l of an aqueous solution containing 66.5□g of ^{14}C-Si-QAC (4□g cm^{-2}) was applied with a syringe evenly over the entire demarcate skin area. Immediately after the dermal application, the surface was dried quickly by gentle air blowing. To prevent possible oral ingestion or wiping off the applied compound, the entire back was covered by wrapping the trunk of the animal with a cotton cloth (collar), taped to the hair for the entire study period. The test material remained in contact with the skin for 10 days. For i.v. administration, a saline solution of ^{14}C-Si-QAC (69.4 □g per 100□l) was slowly injected into the marginal ear vein of the rabbit. Immediately after treatment, animals were transferred to their individually marked stainless steel metabolism cages and maintained on a standard laboratory rabbit ration. Food and water were provided *ad libitum*.

The method of urine and feces collection was the same for both i.v. and dermal application experiments. All urines and feces were collected at 24h intervals for 10 days, except the first day when urine was collected for a 12h time period, and frozen until analyzed for ^{14}C content.

Immediately after obtaining the last urine and fecal samples, the animals were anaesthetized with methoxyflurane and killed by withdrawing blood from the abdominal aorta. Plasma was separated by centrifugation. The total skin application sites were excised. Whole liver, kidneys, lung, spleen brain, gonads, samples of skeletal muscle (from the hind leg) and peritoneal fat were removed, weighed and frozen until analyzed for total ^{14}C determination. The brain was macerated before taking samples for ^{14}C content.

Determination of Radioactivity

Preparation of Samples. Duplicate urine and plasma samples (1.0ml) were pipetted into labeled scintillaiton vials containing 15ml of Aquasol and mixed thoroughly prior to counting. Feces were dried and homogenized in a blender. Duplicate samples of homogenate (approximately 250mg) were combusted in a Sample Oxidizer (Harvey Instruments, model OX-300). The recovery efficiency of ^{14}C (when feces were combusted) was over 97%. ^{14}C-Carbon dioxide was trapped in scintillation vials containing 15ml of monoethanolamine and Dowanol7 EM (3:7, v/v).

The entire dermal application site was dissolved in 50ml of Protosol and duplicate samples (100 µl) of digested skin were counted after the addition of 15 ml of Econofluor. Representative duplicate samples (100-200 mg) of each tissue were transferred to labeled scintillation vials containing 1.0 ml of Protosol. The vials were placed in an oven for 18h at a temperature ranging from 55 to 60°C. Samples which were not completely digested during this time were again kept at 55-60°C for 18h after the addition of 0.5ml of Protosol. Fifteen ml of Econofluor7 was then added to each digested sample. To insure homogeneity, methanol (0.2-2.0ml) was added to samples which contained any precipitate and haziness.

The portion of the cotton collar that was in contact with the site of the dermal application as easily located with the use of a Nuclear Chicago Survey Meter (Geiger-tube type). This area was excised from the collar and dissolved in 70% sulfuric acid (50ml for every 3-5 g sample) and then agitated for 2h at 37°C. Duplicate samples (100µl) of dissolved portions of the collar were diluted in 2.0 ml of distilled water before adding 15ml of Aquasol.

To ensure maximum recovery of the test compound, the remaining portion of the cotton collars were extracted individually with 100ml of concentrated hydrochloric acid and duplicate samples (1.0 ml) were diluted with 1.0 ml of distilled water before adding 15ml of aquasol.

Measurement of Radioactivity. Measurement of ^{14}C was carried out in a liquid scintillation spectrophotometer (Tracor Analytic, Model 6892). The channels ratio method of efficiency calculation was used. Several samples were crosschecked for efficiency by the addition of an internal standard (^{14}C-toluene, $4.5x10^5$ dpm ml^{-1}, New England Nuclear, Lot 697-242). An external standard channels ratio was also obtained. Counting efficiencies for samples were in the range 75-94%. No counts were recorded until the samples had stabilized (dark and cold adaptation) and all samples were checked for homogeneity. The total counts used in calculations were at least twice background levels.

RESULTS

No signs of systematic toxicity were observed among the animals of either group and no chemical-related gross pathological alterations were seen in any organs or tissues examined at the time of necropsy.

TABLE I

Recovery of radioactivity after the administration of ^{14}C-SI-QAC to Rabbits

Radioactivity recovered (mean ± SD)[a]

	Day	Intravenous	Percutaneous
Urine	1	1.38±0.54	–[b]
	2	0.68±0.40	–
	3	0.34±0.44	–
	4	1.06±1.04	–
	5	0.78±1.20	–
	6	1.26±1.52	–
	7	0.58±0.54	–
	8	1.30±1.12	–
	9	1.12±0.90	–
	10	0.86±0.97	–
	Cumulative	9.36±7.64 (13.5±11.0)	0.00
Feces	1	1.54±0.96	–
	2	2.54±1.50	–
	3	1.84±1.21	–
	4	1.52±0.84	–
	5	2.44±0.56	–
	6	0.94±0.80	–
	7	1.30±1.26	–
	8	1.26±1.42	–
	9	0.44±0.59	–
	10	-	–
	Cumulative	13.82±4.25 (19.9±6.1)[a]	0.00

[a] Total recovery of radioactivity of unchanged ^{14}C-Si-QAC for 10 days presented as μg equivalents per total volume of urine or per total dry weight of feces. Values in parentheses are expressed as per cent of the administered dose.

[b] Not detected.

The amount of radioactivity excreted in urine and feces over a period of 10 days after intravenous and dermal administration of ^{14}C-Si-QAC is reported in Table 1. The cumulative 10-day urinary excretions of ^{14}C-radioactivity in rabbits after i.v. and dermal administration were 13.5% and 0.0%, respectively. The excretion of radioactivity in the urine after i.v. administration was well spread over the entire 10-day study period and no particular trend was observed, thereby suggesting the retention of the test compound some way in the organs or tissues. The cumulative 10-day fecal excretion of ^{14}C radioactivity after i.v. and topical administration was 19.9% and 0.0%, respectively. As evidenced by the urinary and fecal excretory values in the i.v. treated group, the total fecal excretion of ^{14}C radioactivity was approximately 1.5 fold greater (19.9± 6.1 vs. 13.5 ± 11.0) than that in the urine.

Tissue distribution of radioactivity after i.v. and topical application of ^{14}C-Si-QAC is presented in Table 2. The liver, lung and kidneys, organs involved in the elimination of substances, showed most of the radioactivity among the isolated organs and tissues of animals killed 10 days after i.v. administration of the test compound. Radioactivity in other organs, tissues and plasma was not detected. The highest ^{14}C concentration was observed in the liver followed by those in the lungs and kidneys. All of the organs and tissues of dermally treated animals showed no concentration of ^{14}C derived radioactivity. Using the Feldmann and Maibach method of calculation, [3,4] the percutaneous absorption of

organosilicon quaternary ammonium salt through the skin of rabbits was essentially zero. Most of the topically applied radioactivity (82%) was recovered from the skin and the cotton collar (Table 3).

TABLE II

Concentration of radioactivity in organs and tissues 10 days after the administration of ^{14}C-Si-QAC to rabbits

	Distribution of radioactivity (mean±SD)	
Organ or Tissue	Intravenous	Percutaneous
Liver	9.52±2.82	–
Kidney	1.86±0.64	–
Lung	1.90±1.25	–
Spleen	0.20±0.07	–
Gonads	[b] –	–
Brain	–	–
Fat	–	–
Skeletal muscle	–	–
Skin	–	27.72±7.30
Collar	–	26.72±7.10
Total[a]	13.4±4.4 (19.4±6.4)	54.44±2.80 (81.90±3.80)

[a] Total concentration of radioactivity of unchanged ^{14}C-Si-QAC expressed as µg equivalents per wet weight of organs or tissues. Valued in parentheses are presented as percent of the administered dose.

TABLE III

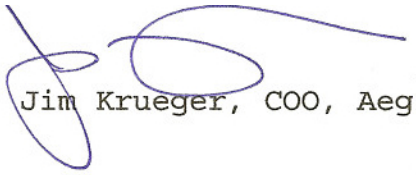
Total recovery of radioactivity in urine, feces, tissues and cotton collar after the administration of ^{14}C-Si-QAC to rabbits

	Radioactivity recovered (% of dose)[a]	
Parameter	Intravenous	Percutaneous
Urine	13.5±11.0	[b] –
Feces	19.9±6.1	–
Organ/tissue	19.4±6.4	–
Skin		41.7
Collar		40.2
Total	52.8±11.9	81.9±3.4

[a] Values represent mean ± SD.
[b] Not detected.

The recovery of a labeled compound=s radioactivity in the urine following i.v. administration provides an estimate of urinary excretion of the compound or its metabolites once it has penetrated the skin and entered the systemic circulation. To calculate the percutaneous absorption, the percentage of applied radioactive dose recovered in the urine following dermal application is divided by the amount of the radioactive dose recovered in the urine following i.v. administration. As expected the estimate absorption of ^{14}C-Si-QAC through the skin of rabbits was essentially zero. The absence of ^{14}C-derived radioactivity in the organs, tissues, and fecal excretions further support the results that the ^{14}C-Si-QAC had not penetrated the skin of rabbits.

[b] Not detected.	

Signature: 

Jim Krueger, COO, Aegis Environments

The first phase of percutaneous absorption is the diffusion of the chemical through the stratum corneum, the rate limiting barrier of the skin.[7] The diffusion rate of a chemical is directly related to the lipid solubility and inversely related to the molecular weight.[8] Si-QAC, with a molecular weight of 498.5, reacts with water to form higher molecular weight polymers. In the polymerized form, Si-QAC is composed of a series of cationic nitrogen moieties combined in a three-dimensional matrix. The cationic charge, lipid insolubility, and molecular weight in multiples of almost 500 units are all evidence against dermal absorption.

The poor recovery (53%) of i.v. injected ^{14}C-Si-QAC can be partially explained by the fact that ^{14}C-Si-QAC is a reactive chemical and tightly bonds with surfaces; it is very likely that some of the radioactive material might not have reached the blood circulation and was lost to the needles, syringes or application sites. It is also possible that some of the ^{14}C radioactivity might have been lost in the expired air since we neither monitored the radioactivity in the expired air nor assessed it by whole body counts.

In view of the present results, it is concluded that this organosilicon quaternary ammonium chloride is not absorbed through the skin of rabbits. The potential hazard of the use of such microbial agents in contact with the skin is therefore considered to be insignificant.

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