UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

DATE: APR 23 1982

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Registration of Requat brand Anti-microbial 1977 Liquid for Industrial use (application to textile fabrics) Reg. No. 46620-R Acc. No. 246194 CASWELL #331C
Di-n-decyl methyl (3-trimethyl oxysilylpropyl) ammonium chloride

FROM: Henry Spencer, Ph.D. 4/16/82
Review Section #1
Toxicology Branch/HED (TS-769)

TO: John Lee, PM #31
Registration Division (TS-767)

THRU: Robert B. Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769) 4/16/82 4/23/82

SIGN HERE

Recommendations and Conclusions:

1. TOX Branch considers the registration for industrial use toxicologically supported.

2. The concentration of Methanol in the material (50%) requires the signal word, POISON, on the side label. The concentration of quaternary ammonium compound (45%) is also known to be corrosive to the eyes. (Tox Cat. I) Therefore, the label should be changed from "avoid" repeated, to: "do not get in eyes, on skin or clothing".

3. The requested uses listed in the technical bulletin are not totally supportable; socks and towelling are not supportable. TOX Branch finds that only the uses on mattress ticking, mattress pads, non-woven fabrics, and shoes 4/23/82 4/23/82 are supportable by the data submitted with this registration request since the likelihood of significant exposure is low. Additional data will be necessary to support the requested uses on socks, towelling or other fabrics which may remain in intimate contact with the skin.

4. Repeated leaching data are required taking into consideration the attachments #1, "Points to remember in a leaching study of Fabrics".

5. The registrant requested at our meeting on March 16, 1982, a clarification of the protocol submitted Jan. 11, 1982. Dermal Uptake of a Quaternary Amine Pesticide. TOX Branch has changed the submitted protocol in an attempt to follow this request. Attachment #2.

6. No RPAR criterial have been exceeded.

7. Currently lacking data include.

   a. Adequate leaching data.
   b. A ^{14}C dermal absorption study if the leaching data are positive for leaching potential.
   c. Long term oncogenicity, mutagenicity, teratogenicity reproduction studies may be necessary if the dermal absorption study shows significant effects.

Review:

Acute Oral Toxicity, Single Dose LD_{50}

Test completed by: United States Testing Company, 1415 Park Ave., Hoboken, New Jersey 07030
Test Material: Compound 1977. (technical)
Study Number: 02266-1 dated 11/21/77
Acc. No.: 246194
Methods: Groups of ten Sprague Dawley rats (5 per sex) weighing 185-220 gm were gavaged after 24 hr. removal from food. Water was available ad lib. Observations were made at 1 hr., 4 hr. post gavage and daily for 14 days. The method of calculation was that of Litchfield and Wilcoxon.

Dose	Dead/dosed
11 ml/kg	1/10
13 ml/kg	4/10
15 ml/kg	7/20
16 ml/kg	5/10
17.5 ml/kg	8/10
20 ml/kg	7/10

Observations: Malaise and anorexia, most mortality in 24 hrs.

Results: LD_{50} = 15.5 ml/kg (95% CL; 13.83-17.36)
Core Evaluation: Minimum data

Acute Dermal Toxicity:

Test completed by United States Testing Co. 1415 Park Ave., Hoboken, New Jersey 07030.

Study No: 02266-3 Date: 12/6/77

Test Material: Compound "1977"
Methods: New Zealand albino rabbits were used weighing 2.0-2.7 kg. The sex of the animals were not stated. Flanks and backs were shaved free of hair. One half the animals were abraded over their backs. Dosages were 10 ml/kg and 15 ml/kg.

Results:

0/5 animals died at the 10 ml/kg dosage.
4/4 diet at the 15 ml/kg dosage.

Observations:

Neurological effects - loss of coordination lapsing to stupor and comatose condition, skin effects "leathery skin".

An LD_{50} occurs at > 10 ml/kg <15 ml/kg. (though a finite LD_{50} has not been calculated)

Tox. Cat. III
Core Minimum Data

Skin Sensitization Study
Guinea Pig Assay

Test completed by United States Testing Company Inc. 1415 Park Avenue, Hoboken, New Jersey 07030.
Dated: Dec. 5, 1977. #02266-2
Acc. No. 246194
Test Material: "Compound 1977"
Test Annual: Albino Hartley Guinea Pigs 250-300g.
Methods: repeated, occluded topical patch technique. Hair was clipped from either backs or flank.

Induction was carried out by 1% or 10% dilutions in H_2O on 1 x 1 inch gauze pads (occluded). Six (6) received the 10% dose and (4) received the 10% and a 1% dose on their backs. Exposure was 48 hrs.

The second induction period occurred 1 week later as a repeat exposure.

Challenge: 1 week after 2nd induction.

Non-irritating doses were applied to new sites using both 1% and 2% solutions and occluded for 24 hrs. Evaluation occurred immediately after application removal and at 1/2, 4, 24, and 48 hrs. later.

A second challenge 1 week after 1st challenge using an open topical application of a 1% solution of "1977" and observed at 1, 4 and 24 hr. Results: only slight irritation occurred with the 10% solution in the 2 induction periods. No allergic response occurred in challenge phases.

Comment:

The study did not reference the protocol or why such abbreviated exposure period and rest periods were appropriate.

Exposure and rest periods are considered to be so brief as to question ability of the study to display any but a negative reaction.

Core Evaluation:

Supplementary-not sensitizing under the conditions of the study. May be elevated to minimum, if data is presented which indicates that this method is capable of detecting a known human sensitizer.

Study: Evaluation of Potential Hazards by Dermal Contact.
Acc. No. 246194

Test Material: 50%-50% cotton, polyester blend treated with 2%-3% "compound 1977". Completed by Product Investigations Inc. 151 E. 10th Ave. Conshohocken, Pa. 19428. Date: Feb. 22, 1978. Study No. PI 19-05-311.

Test Animals: Humans, volunteers.

Methods:

The test material was moistened with tap water and applied to the forearms of the volunteers and sealed with tape. Observations occurred after 24 hr, the skin was rested for 24 hr and reexposed for 24 hrs., etc.

After the 15th exposure, the volunteers were rested for 2 weeks and challenged. Challenge occurred in the same manner as the induction exposures.

Results:

No reactions occurred in any of the test sites.

Core Evaluation: Minimum Data.

This reviewer agrees with the investigator, in that the material is not likely to cause irritation, fatiguing or sensitization but only under conditions not exceeding those of the study.

Study: Leaching study of Requat® Silicon from treated fabrics.

Study By: William Roessler-received at EPA dated 10/20/81.

Acc. No.: 246194

Materials:

65%/35%, polyester/cotton
100% nylon (Banlon, Tex nylon)
cotton (mercerized 80/80).

Materials were Examined as:
(Blend) 28 Jan., 4 May

1. Untreated and unwashed
2. 1% treatment, unwashed
3. 3% treatment, unwashed
4. 1% treatment, washed 10X
5. 3% treatment, washed 10X
6. untreated, washed 10X

100% Nylon
100% cotton 10 July

Treated at:

1. 1%
2. 2%

Washing was done with Ajax detergent and deep well water in a porcelin tub washing machine. After being washed, unwashed, treated or untreated, 2.5 gm swatches were auto claved and leached in a rotory shaker at 36°C for 24 hr. in 50 ml aliquots of:

a. phosphate buffered saline Ph 7.2
b. synthetic sweat
c. ultrafiltered human urine
d. blood plasma 1:1 saline (a).

After leaching, the solutions were filtered and analyzed for silicon at the University of Maryland by D.C. plasma emission spectroscopy. (Sensitivity = 0.1 ppm)

Results of Silicon Analyses (First Series & Second Series) Blend
(65/35 polyester-cotton bleand)

A. Buffered saline pH 7.2

	Silicon mg/L
Control (saline)	0.71
Untreated washed	4.09
1% treated, unwashed	5.44
3% treated, unwashed	14.53
1% treated, washed	12.6
3% treated, washed	19.6

* missing untreated unwashed ?

B. Synthetic Sweat

	1st set		repeat washings
Control (synthetic sweat)	0.15	ND	
Untreated, washed	5.64	8.6	8
1% treated, unwashed	6.97	--	
3% treated, unwashed	17.27	23	0
1% treated, washed	19.20	--	
3% treated, washed	22.90	26	10
untreated unwashed		0.3	
detergent		75.0	
well water used in home laundry		10.0	0

C. Blood Plasma & Buffered Saline

Control (1:1)	0.65
Untreated, washed	4.50
1% treated, unwashed	11.26
3% treated, unwashed	30.7
1% treated, washed	23.3
3% treated, washed	22.3

Results: Third Series

Nylon (100%)
Cotton (100%)

			mg/L
Control (synthetic sweat)			0.25
Nylon		Cloth	
untreated	washed	0.5g	0.27
untreated	washed	2.5g	0.32
1% treated	washed	0.5g	0.94
1% treated	washed	2.5g	3.4
2% treated	washed	0.5g	2.2
2% treated	washed	2.5g	10.0
Cotton			
untreated	washed	0.5g	2.1
untreated	washed	2.5g	9.1
1% treated	washed	0.5g	1.3
1% treated	washed	2.5g	5.2
2% treated	washed	0.5g	2.1
2% treated	washed	2.5g	8.8

Urine was found to have from 34.7-37.6 mg/l of silicon.

Discussion:

1. The use of real urine to leach the fabric is inappropriate due to the high (34-38 mg/l) levels of silicon present in the fluid.

2. The use of synthetic sweat in the leaching study indicates that only 0.3 mg/l was present in the polyester blend leachate without treatment or washing. However washing, alone will cause the material to be contaminated i.e. 5.64 mg/l and 8.6 mg/l leached with no treatment, washing and leached. Data are not present to discern whether the 1%, 2% and 3% treatment followed by washing before leaching exhibit a supposed leachate contamination from the "quat" or from the "quat" gaining Silicon from the washing products.

The 3% treatment however, does exhibit an approximate 3 X increase when not washed but leached. The differences between treated unwashed and treated washed materials is variable but again suggests that the washed material gains Silicon from some source and later is leached off the fabric. Quantitatively the data is not adequate to delineate a value of leached silicon.

Invalid - Controls were deleted.

The study does not cover a pH range of that found with regard to the human body situation, and is required for an evaluation of fabric use under those circumstances.

ATTACHMENT 1

Points to remember in a leaching study of Fabrics

The leaching study should use distilled or deionized water in all phases with very low levels or no silicon present.

All fabric samples should have untreated and unwashed samples leached as controls.

Swatches should be the same size through out; leaching solutions should cover the pH range of from 4.5 to 9.0 and should also include an intermediate value of 7.0 to 7.5. Containers to wash, or leach the swatches should be made of materials low or devoid of silicon. A liquid detergent, liquid soap, or other cleaning material nearly devoid of silicon fillers should be used in the washing phase of the study.

Washing can be accomplished in 1-2 liter nalgene screw-cap bottles. Final air drying prior to leaching is acceptable to preclude contact contamination with silicon.

A shaker set at very low speed (no more than 30/minute) for the leaching phase at room temp. should be used. The leachate should be centrifuged in poly proplylene or other types of non silicon containing containers i.e. 20,000 to 40,000g for 20 minutes to remove fibers, decanted and refrigerated for silicon analysis.

ATTACHMENT II

Dermal Uptake of a Quaternary Amine Pesticide

I. Introduction

This protocol is adapted from the one provided by the staff of the Toxicology Branch, Registration Division, Environmental Protection Agency for pesticides that are relatively toxic by the dermal route. Of particular concern was the dermal absorption of the pesticide by the occupationally exposed individual; additionally, the data derived from the test was necessary for the risk assessments associated with the establishment of reentry intervals when the pesticide is used in the field. The quaternary amine to be tested by the following protocol is not to be used as an agricultural pesticide; its toxicity is much less that those used in field situations. It is to be impregnated into fabrics as a pesticide to inhibit the growth of microorganisms on the fabric. Of concern to the Agency are the toxicological implications of treated fabrics that come in contact with human skin.

The investigation is mainly concerned with the rate of dermal uptake of pesticides by both sexes when exposed to differenct concentrations of pesticides as a model for the dermal uptake of pesticides by man.

According to Feldman and Maibach (1,2) and recently by Wester et al., (3,4), percutaneous absorption can be quantitated on the basis of radioactivity excreted (percent in the urine) over five to ten days following the application of a known amount of radio labelled compound on the skin. To correct for excretion of radioactivity by other routes and retention of radioactivity in the body, urinary-excretion data obtained after topical application of the compound are adjusted in accordance with the urinary excretion observed after intravenous dosage.

II. Materials and Methods:

^{14}C-Labelled pesticide with known specific activity will be used. The radioactive carbon should be so located that it is not removed during the biotransformation process. In the case of the quaternary amine, the radioactive label most appropriate should be incorporated in the chain rather than in the more easily removed methoxy group.

Sprague-Dawley rats, five weeks old of both sexes normally will be used. If only one sex is used, it is preferred that they be females. Eighteen to twenty-four animals/dose level should be used in this experiment.

III. Experimental Procedure:

A. Preparation of Animals:

Weighed and housed individually in stainless steel metabolic cages. The animals will be acclimated for at least one week prior to testing. One day before the commencement of the study, the animals may be anesthetized with ether before treatment. The exposed skin will be washed with a mild detergent to remove dirt, well rinsed with water and dried-after the hair on their back has been clipped carefully without causing abrasion to the skin. A neoprene rubber template (9-16 cm^2) will be glued to the back of the animal with cyanoacrylic glue according to the procedure of Knaak et al. (5). Food and water will be provided ad libitum.

B. Application of Test Material:

Two dosage levels will be tested (9-12 animals each) by treating topically with a water solution of the ^{14}C labelled material. The test chemical will be applied to the skin evenly with a micropipet. Evaporation from the skin is enhanced by gentle heat and/or ventilation. The area of dermal application should remain constant for all experiments. The site of treatment will be covered with a taped cloth cover. The site of dermal treatment should not be occluded unless necessary. The amount of solution placed on the skin should be small, 0.1 to 0.05 ml; thus, the specific activity of the radioactive compound should be high.

C. Sampling:

Urine, feces and blood samples will be collected ever 12 hours after dosing the first day, then every 12-24 hours thereafter for a period of at least en days. Duplicate blood smaples can be easily obtained from the tail by cutting the tip of the tail and bleeding the animal (4 to 5 drops) in a preweighed ^{14}C-oxidizer paper cup containing an absorbent pad. The tail will be treated with molten wax (55° C) and then dipped in cold water to stop bleeding. Blood samples may also be collected in preweighed scintillation vials. In both cases the container has to be reweighed immediately after obtaining the blood samples. All samples and collected organs, will be kept frozen until analysis.

D. Sacrifice and Tissue Sampling

Individual organs may be required for later analysis; therefore, the animal should be anesthetized using methoxyfurane and killed by withdrawing blood from the abdominal aorta in heparinized syringe. The cloth cover will be removed without contaminating the rest of the skin, and the skin at the site of application will be excised for ^{14}C counting. The cloth cover and the excised skin will be kept in two separate jars. Animals will be dissected and the desired organs, at a minimum the liver, spleen, brain, kidneys and gonads, removed, trimmed and weighed. The tissues and organs will be kept frozen until analysis.

E. Sample Processing and Radioactivity Determination:

Duplicate, well mixed, urine samples will be transferred to scintillation vials and radioactivity will be determined after the addition of a liquid scintillation cocktail capable of incorporating aqueous samples.

A homogenate of feces will be prepared by the addition of a few drops of water, weighed, and duplicate samples will be taken in preweighed ^{14}C-oxidizer paper cups and reweighed to determine the exact weight of the samples. Samples may be left to dry in the fume hood before combustion or can be directly combusted in a ^{14}C-oxidizer and then counted, or solubilized and counted.

Blood samples can be directly combusted and counted.

Homogenates of individual tissues should be prepared if the counts in the blood, urine and/or feces samples indicate that appreciable amounts of the pesticide crossed the skin barrier. Duplicate samples can be either digested or combusted and then counted.

Excised skin of individual animals can be washed two times in the same container using acetone, and the washings will be combined. Duplicate samples of the acetone extract will be counted. The residual skin will be cut into four approximately equal pieces and combusted. The count from the four pieces will added together after subtracting the background count and the total will be considered as the amount of residual radioactivity in the skin.

The cloth cover will be extracted three times with acetone and the three extracts will be combined. Duplicate samples will be counted.

All samples need to be dark-adapted before counting. Counts need to be corrected for any quenching using internal or external standards. Background and efficiency have to be determined routinely. Control samples from an untreated animal need to be included each time to determine the background and quenching.

F. Data Reporting:

Records should include the following:

1. Animal number, sex, weight, age, and dose level.

2. Total radioactivity in each sample as percent of the total dosage applied.

3. Raw data including sample weight or volume, total weight of organs, count per minute, background count, counting efficiency and disintergrations per minute. Control animal counts, etc. shall be included.

4. Details of methodology and any modification or the method with an acceptable rationale to justify this modification.

5. Material balance.

G. References:

1. Feldmann, R.J., and Maibach, H.I. Percutaneous penetration of steroid in man. J. Invest. Dermatol. 52:89-94 (1969).

2. Feldmann, R.J., and Maibach, H.I. Absorption of some organic compounds through the skin in man. J. Invest. Dermatol. 54: 339-404 (1969).

3. Wester, R.C., and Maibach, H.I. Percutaneous absorption in the rhesus monkey compared to man. Toxicol. Appl. Pharmacol. 32:394-398 (1975).

4. Wester, R.C., Noonan, P.K., and Maibach, H.I. Recent advances in percutaneous absorption. J. Soc. Cosmet. Chem. 30:297-307 (1979).

5. Knaak, J.B., Schlocker, P., Acherman, C.R., and Seiber, J.N. Reentry research: Establishment of safe pesticide levels on foliage. Bull. Environ. Contam. Toxicol. 24:796-804.