J. Soc. Cosmetic Chemists, **18**, 527–536 (Aug. 19, 1967)

Deposition of Hexachlorophene on the Skin

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Presented November 30, 1966, New York City

Synopsis—Colorimetric analyses of alcoholic extracts of the skin were used to determine the hexachlorophene content of the skin after washing with hexachlorophene-containing soaps. The quantity of hexachlorophene applied was found to be a major factor controlling the amount deposited. Significant quantities of hexachlorophene were left on the skin after soaking in baths containing very low concentrations of the compound.

Introduction

Hexachlorophene has been widely used as the antibacterial component in degerming soaps and detergent formulations. The reduction in the number of bacteria on the skin achieved through the continued use of these products is well documented (1). It is attributed to the buildup of effective levels of the compound on the skin (2). However, determination of these levels has been the subject of very few publications (3–5). The object of this study was to follow the concentrations of hexachlorophene retained by the skin under controlled washing conditions.

Experimental

Extraction and Analytical Procedures

The forearms were selected as the test site since they were accessible, maneuverable, and less exposed to contamination than the hands. The hexachlorophene was recovered from the skin by immersing the arm,

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Table I
Recovery of Hexachlorophene

Vehicle	Quantity applied (mg)	Concentration found (mg)
Alcohol	0.50	0.52
Alcohol	0.50	0.53
Alcohol	0.50	0.45
Alcohol	0.50	0.46
Soap	0.50	0.56
Soap	0.50	0.57
Soap	1.00	1.04

from wrist to elbow, in a vessel containing 1000 ml of 95% ethanol while gently agitating the solution. The alcoholic extract was concentrated on the steam bath to a volume (10 to 100 ml) suitable for hexachlorophene analysis.

The 4-aminoantipyrine colorimetric procedure was selected as the most suitable method for determining the hexachlorophene in the concentrated extract. The ultraviolet absorption method, with which this laboratory has had extensive experience, was ruled out. The levels of hexachlorophene were low in many cases, and the UV absorption by skin extracts frequently swamped the absorption by the hexachlorophene.

An ammonia buffer solution (6) was used in place of sodium carbonate described in the literature (7, 8). 4-Aminoantipyrine forms a red color with hexachlorophene in the presence of a mild oxidizing agent. The color density was measured with a Beckman DK2 automatic recording spectrophotometer. The entire absorption curve was scanned so that interferences or turbidity could be observed.

Preliminary tests with known amounts of hexachlorophene applied to the skin were conducted to determine the relative validity of these procedures. The hexachlorophene was distributed over the forearms as alcoholic or 5.0% liquid soap solutions and permitted to dry. The arms were then immersed in alcohol, and the extract was condensed to 10.0 ml for analysis. Results of these tests (Table I) demonstrate that quantitative recovery and analyses of the hexachlorophene content of the skin could be made with these procedures.

Soap Bar Tests—Single Applications

The forearms were washed with soap bars ($1\frac{1}{4} \times 2\frac{1}{4}$ in.) containing hexachlorophene using the following general procedure:

Table II
Single Washing

Hexachlorophene Concentration in Soap (%)	Hexachlorophene Concentration on Skin (γ/cm^2)
0.25	0.15, 0.29, 0.31, 0.32
0.50	0.42, 0.42, 0.45, 0.47, 0.47
1.0	0.52, 0.65, 0.74, 0.81, 0.82 0.82, 0.84, 0.90, 0.92, 1.02
2.0	0.90, 0.95, 1.02, 1.05 1.09, 1.19, 1.19, 1.29 1.34, 1.35, 1.42, 1.47
5.0	1.94, 2.74, 3.39, 3.55 3.55, 3.71, 4.03, 4.35

Plastic gloves were fastened on both hands, the arms were moistened with warm water, and the test soap was applied by rubbing all areas of the forearm from wrist to elbow for fifteen seconds. The arm was then lathered for thirty seconds and rinsed with 1500 ml of warm water. Immediately after rinsing, the arm was immersed in 1000 ml of alcohol and the extract condensed and analyzed as previously described. Most of the tests in this investigation were conducted on the forearms of the same two subjects. Occasionally, additional subjects were included for a single test and in every instance produced comparable data to the standard subjects.

Results of single washings with bars containing various concentrations of hexachlorophene are listed in Table II. Each figure represents the quantity recovered from an arm after one washing with the specified soap bar. The data are recorded as micrograms hexachlorophene per square centimeter of skin, using 620 sq cm as the approximate area of forearm skin. A minimum period of forty-eight hours was permitted to elapse after each test before an arm was re-used. These results show that the higher the hexachlorophene content of the bar the greater the deposition on the skin. Despite some spread in the results for a given soap and the slight overlapping between the 1 and 2% bars, each of the test soaps can be differentiated from another by the quantity of hexachlorophene deposited.

The amount of hexachlorophene retained by the skin from the various soaps was directly compared in a series of repetitive tests. Different arms were washed at the same time with bars containing 1, 2, and 5% hexachlorophene, and the quantity left on the skin was determined.

Table III
Single Washing—Replicate Tests

Hexachlorophene Concentration in Soap (%)	Hexachlorophene Concentration on Skin (γ/cm^2)				
	Test 1	Test 2	Test 3	Test 4	Test 5
1.0	1.00	1.15	0.81	0.63	0.77
2.0	1.55, 1.42[a]	1.65, 1.47[a]	1.40	1.08	1.50
5.0	2.90	3.03	2.60	2.32	2.58
Blank	...	...	...	0	0

[a] Two arms tested with 2.0% hexachlorophene soap.

Table IV
Multiple Washing—4 Day Period

Hexachlorophene Concentration in Soap (%)	Hexachlorophene Concentration on Skin (γ/cm^2)
1.0	1.7 (0.82)[a]
2.0	3.5 (1.19)
5.0	9.4 (3.55)
10.0	15.5

[a] Numbers in parentheses are medians of results obtained from one washing (Table II).

This test was repeated four times on four different days. At least forty-eight hours lapsed between tests. Results in Table III show that within each test run there is an increase in hexachlorophene deposition as its content in the soap increased. However, this relationship is not necessarily present if isolated data from one test are compared to another; for example, the 1% bar in Test 2 deposited more than the 2% bar in Test 4. This discrepancy apparently stems from the fact that all of the test soaps deposited their lowest quantities in Test 4 and their highest in Test 2. This is illustrated in Fig. 1. Therefore, the comparative effects of different soaps is best determined by tests conducted at the same time.

Multiple Washings—Four-Day Periods

Tests were conducted in which the arms were washed with soap bars six times over a four-day period and the hexachlorophene retained by the skin determined after the sixth application. These results, listed in Table IV, show a significant increase in the quantity deposited by all bars compared to a single application (see Tables II and III).

Multiple Washings—One-Day Period

Previous investigations (4) had demonstrated that the quantity of hexachlorophene retained by the skin reached a "plateau" level after a number of daily washings and remained relatively constant thereafter. An attempt was made to attain a plateau level in a single day by washing the arms with a soap and rinsing every twenty minutes. The tests were conducted with both 1.0 and 2.0% hexachlorophene-containing

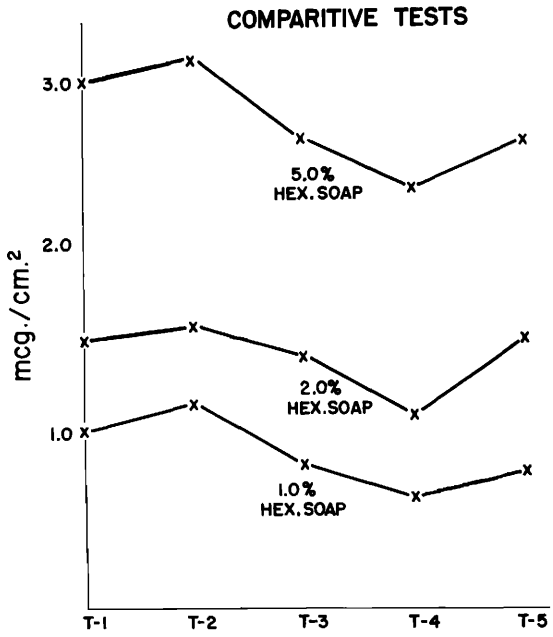

Figure 1. Deposition of hexachlorophene on skin from soaps

soaps. Different arms were immersed in alcohol, the hexachlorophene was extracted after the ninth, twelfth, sixteenth, and twentieth washings, and the quantity deposited was determined. The data in Table V show an irregular but continual rise in the hexachlorophene buildup on the skin as the number of washes increased. No plateau effect could be demonstrated in these tests. The quantity of hexachlorophene retained from the 2.0% bar was approximately one and one-half to two times the amount retained from the 1.0% soap through the twenty applications.

Table V
Multiple Washing—1 Day Period

Number of Washings	Concentration on Skin (γ/cm^2) 2.0% Hex. Soap	1.0% Hex. Soap
9	3.2	1.8
12	3.9	1.9
16	4.0	2.3
20	4.7	2.6

Table VI
Single Washing—Extended Time Periods

Length of Time of Soap Application (minutes)	Concentration of Hexachlorophene on Skin (γ/cm^2)
0.25	1.3
2.0	2.3
4.0	3.6
8.0	5.2

Single Application—Extended Washing Time

The accumulation of hexachlorophene on the skin was measured using a single washing with varying time periods for the application of the soap. The arms were washed with a 2.0% hexachlorophene soap for periods up to eight minutes, rewetting the soap every two minutes. The arms were then lathered and rinsed, and the hexachlorophene was extracted in the usual manner. Analysis of these extracts, listed in Table VI, clearly indicated a marked increase in the amount of hexachlorophene left on the skin with increased time of application of the soap.

Bath vs. Shower Tests

Skin retention tests were conducted comparing a shower to a bath rinsing procedure. Exactly 1.0 g of a 2.0% hexachlorophene soap bar was applied to each of the forearms with frequent intermittent rinsing until the entire soap sample had been consumed. Rinsing by shower was accomplished by spraying warm water from a shower head over the arm. The bath rinse consisted of immersion of the arm in a pan containing 3000 ml of water with a final four-minute soaking in this

water. Analysis of the alcohol extractions of the arms prodcued the following results:

	Concentration of Hexachlorophene on Arms	
Procedure	Total (mg)	γ/cm^2
Shower	1.34–1.66	2.2–2.7
Bath	1.48–1.56	2.4–2.5

These data show that approximately equivalent amounts of hexachlorophene are retained by the skin from both procedures. It is interesting to observe that the skin retained about 7.5% of the total hexachlorophene applied (20.0 mg in 1.0 g of 2.0% hexachlorophene soap).

Bath Tests

During these studies it was found that significant quantities of hexachlorophene would be retained by the skin after immersion in a bath containing low concentrations of the compound. Aliquots of a 10.0% hexachlorophene solution in 50% ethanol were added to a bath containing 5000 ml of warm water. The forearm was moistened in the bath, washed with a nonmedicated soap, then reimmersed in the bath for a period of five to ten minutes. Immediately after bathing the arms were immsered in alcohol; the extract was condensed and analyzed for hexachlorophene. The results are listed in Table VII with each of the figures representing an individual test conducted on a single arm in a bath with the specified hexachlorophene content. The data clearly demonstrate that substantial quantities of hexachlorophene are deposited on the skin from baths containing relatively low concentrations of the compound. Bathing in water containing 4.0 mg/l of hexachlorophene deposits approximately the same amount of compound on the skin

Table VII
Bath Tests

Hexachlorophene Concentration in Bath (mg/l)	Hexachlorophene Concentration on Skin (γ/cm^2)
4.0	0.87, 0.87, 1.08, 1.26 1.35, 1.61, 1.77, 1.77, 2.10
20.0	3.39, 4.19, 4.35, 5.00 5.65, 5.81
40.0	9.35, 9.35, 10.00

Table VIII
Bath Oil Tests

Product	Hexachlorophene Concentration in Bath (mg/l)	Hexachlorophene Concentration on Arm (γ/cm^2)
Bath Oil "A" (3.0% Hex.)	6.0	4.2–8.2
Bath Oil "B" (4.0% Hex.)	12.0	10.6–17.4
Bath Oil "C" (1.0% Hex.)	3.0	2.7–4.2
Bath Oil "D" (3.0% Hex.)	10.0	5.2–6.8

as a single washing with a 2.0% hexachlorophene soap. Raising the hexachlorophene content of the bath results in an increased deposition on the skin. Several of these tests were repeated without washing with the nonmedicated soap, and analogous results were obtained.

Bath Oils

Skin retention tests were conducted in baths containing various bath oils formulated with hexachlorophene. Results of these tests, listed in Table VIII, demonstrate the relatively large quantities of hexachlorophene that can be put on the skin by use of these products. The wide spread in results for a given product can be attributed to nonuniform distribution of the floating oils through the bath.

Discussion and Summary

Previous studies of hexachlorophene on the skin have employed bioassay (3), radioactive techniques (4), and ultraviolet analysis of alcohol extracts (5). Shemano and Nickerson (4) found that hexachlorophene accumulated on the skin during the first three or four washes and remained relatively constant thereafter. Compeau (5) showed that hexachlorophene builtup during the first five to ten minutes of scrubbing but then accumulated no further after additional washing. He suggested that hexachlorophene was adsorbed on the skin through an ionic reaction with the cationic proteins of the skin. Recently, Parran (9) suggested the analogy between retention of antimicrobials on the skin and the problem of soil redeposition during the laundering of clothes.

This study determined the hexachlorophene content of skin by a colorimetric analysis of concentrated alcohol extracts of the skin. It was found that the quantity of hexachlorophene applied to the skin was a major factor in controlling the amount retained. Increasing quantities were deposited by the following methods:

(a) Raising the concentration of hexachlorophene in the soap
(b) Increasing the number of washes
(c) Increasing the amount of soap applied during a single wash

There was no evidence indicating selective adsorption of hexachlorophene onto the skin, and no plateau levels were attained in any of these tests. In many instances the quantity retained varied roughly in direct proportion to the concentration in the soap or bath. Bath tests indicated no exhaustion of hexachlorophene from the bath, and it appeared that deposition depended on the wet pickup of the skin. Therefore, it is suggested that the deposition of hexachlorophene is due to its physical entrapment on the skin. It is probably retained both as individual particles and solubilized in the soap left on the skin after washing.

The relationship between the quantity of hexachlorophene and the number of microorganisms present on the skin has often been implied but never directly studied. Information on this subject could be obtained in washing tests using the hands for bacterial counts and alcohol extractions of the arms for chemical analysis. It has been assumed during hand washing studies that the hexachlorophene content on a subject's hand is built up to effective antimicrobial levels after several days of washing with the hexachlorophene-containing soap. However, the quantity deposited is dependent on the quantity applied, which in turn will vary with the individual washing habits of the test subjects. Therefore, closer control of the mechanics of the washing process in tests with antimicrobial soaps would be in order. These studies would be accelerated by depositing large concentrations of the compound on the skin immediately through the use of a five to ten minute initial wash with continual reapplication of the test soap.

(Received November 30, 1966)

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