

Percutaneous absorption of Triclosan from toilet preparations

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Synopsis—The absorption of [³H] TRICLOSAN (*Irgasan*[®] DP300) through RAT SKIN treated with SHAMPOO containing 0.05% (w/v), and with AEROSOL DEODORANT containing 0.1% (w/v), has been measured. The products were applied in a manner designed to simulate consumer use, and the penetration was calculated from the amount of radioactivity excreted by the animals. From the shampoo, the penetration was 0.197 $\mu\text{g cm}^{-2}$ which increased as the concentration of [³H] Triclosan was increased but which was independent of duration of contact with the skin for a given concentration of [³H] Triclosan. BLOOD LEVELS at 48 h after treatment were proportional to concentration of applied [³H] Triclosan and for 0.05% (w/v) were less than the equivalent of 0.1 $\mu\text{g ml}^{-1}$. From the aerosol deodorant the penetration was 6.85 $\mu\text{g ml}^{-1}$ and the blood level reached a maximum, equivalent to 0.26 $\mu\text{g ml}^{-1}$, at 6 h after a single application.

The calculated absorption by the human is an extremely low proportion of the no-effect level in rats.

INTRODUCTION

It has previously been established that small amounts of the germicide, hexachlorophene, can penetrate through intact skin (1) and can also be identified in blood, adipose tissue, brain and other body organs (2, 3). The compound 2-hydroxy, 2¹,4,4¹-trichlorodiphenyl ether, now called Triclosan and formerly known as *Irgasan*[®] DP300 (Ciba Geigy Ltd), has some chemical features in common with hexachlorophene and has been shown to be effective in reducing both Gram positive and Gram negative

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bacteria on the skin (4). It has the advantage over hexachlorophene of being of low toxicity (4). Nevertheless, it is important to know the degree to which it could be absorbed through skin from a variety of skin products. We have previously reported on the percutaneous absorption in guinea-pigs of Triclosan from soaps (5) and this present report examines its absorption through rat skin treated with shampoo containing various concentrations of [^3H] Triclosan and with aerosol deodorant containing 0.1% (w/v) [^3H] Triclosan. The possible absorption by the human of 0.05% (w/v) Triclosan in a shampoo and of 0.1% (w/v) Triclosan in an aerosol deodorant is calculated from the rat data.

METHODS

Materials

Tritiated Triclosan was prepared and purified as described by Black, Howes and Rutherford (5) and had a specific activity of 44.86 $\mu\text{Ci mg}^{-1}$.

Samples of [^3H] Triclosan were accurately weighed out and the shampoo base was added to give concentrations of 0.05, 0.1, 0.5, 1.0 and 2.0% (w/v) of [^3H] Triclosan. The mixtures were stirred at 50°C for 1–2 h to ensure complete solubilization of the germicide. Aliquots of each preparation were counted to determine the precise amount of [^3H] Triclosan applied to the rats.

A single can of aerosol deodorant was prepared by dissolving [^3H] Triclosan in ethanol and adding the other ingredients to give a concentration of [^3H] Triclosan in the product of 0.1% (w/v). The can was sealed and the propellant added through the valve from another cannister.

ANIMALS AND TREATMENT

Subcutaneous turnover of Triclosan

Twelve female Colworth-Wistar rats (120 g) were injected with 0.5 ml of [^3H] Triclosan solution in 50% aqueous polyethylene glycol (BDH, Poole, Dorset) under the loose skin over the upper thorax. The animals were placed immediately in the individual metabolic cages for up to 96 h and 24-hourly samples of the separated urine and faeces were collected for determination of tritium. Some rats were killed at 24, 48, 72 and 96 h after being anaesthetized and heart blood taken.

Shampoo application

Thirty-two female Colworth-Wistar rats (110–130 g) were clipped and the exposed lumbar skin was pre-washed with a 20% (v/v) solution of the shampoo base, rinsed and dried. Twenty-four hours later the animals were lightly anaesthetized with cyclopropane, oxygen and carbon dioxide gas mixture, and an area of 7.5 (or 15) cm² of skin was marked. 0.1 ml (or 0.2 ml) of the test shampoo was applied to the skin (7.5 or 15 cm² respectively) and spread over the marked area with a rounded glass rod. 0.1 ml (or 0.2 ml) of water was applied to the marked area and lathered with the glass rod for 1 min. The solution was left for a further 4, 9 or 19 min (5, 10, or 20 min total) in contact with the skin before thoroughly rinsing the skin with distilled water. The rinsings were collected and monitored for tritium and the treated area of skin was dried by lightly dabbing with paper tissues. The treated area of skin was protected with a non-occlusive patch, which was composed of three layers of surgical gauze (3.0 × 3.5 cm for a 7.5 cm² area of skin) covered with 100 mesh stainless steel gauze and the whole wrapped around the trunk of the animal with perforated (5–10 holes/cm²) 'Sleek' tape (Smith & Nephew Ltd). The animals were placed in individual metabolic cages and excreta were collected separately in 24-hourly batches. At the end of the experiment the animals were anaesthetized, terminal heart blood taken and then sacrificed. The protective patch and treated area of skin, which was frozen until required, were monitored for tritium.

Deodorant application

Six female Colworth-Wistar rats (120 g) were prepared as described above. Twenty-four hours later the animals were anaesthetized and a protective card screen with a 7.5 cm² window was placed over the back. The aerosol can was aimed centrally over the window area and a 2-s spray applied with the can held approximately 15 cm away from the skin. The screen was removed, the treatment area marked with a felt-tipped pen and covered with a protective patch. The animals were then placed in separate metabolic cages and treated in the same way as the animals washed with shampoo.

Topical application of Triclosan in ethanol

A solution of [³H] Triclosan in ethanol was applied to the skin of 12 female Colworth-Wistar rats (120 g). The treated area of skin was air-dried for about 20 s after which time the ethanol had apparently evaporated. The treated area of skin was covered with a protective non-occlusive patch

and the animals placed in separate metabolic cages. The animals were killed at various times up to 96 h after application and excreta, blood, skins and protective patches were monitored for tritium.

Rats on all treatments were fed on pellets of *Spital* diet (BOCM/Silcock, Process Development Department, Bromborough, Cheshire) and given water *ad libitum*.

Analysis of biological samples for tritium

Urines were made up to 50 ml and 2.0 ml aliquots were counted in 18.0 ml of *Triton X-100* : Toluene (1 : 2, v/v) liquid scintillator containing 5.0 g PPO and 0.2 g POPOP/l. The samples were thoroughly mixed and counted in a Packard 4322 liquid scintillation spectrometer. A channels ratio technique was used to determine the counting efficiency and [1,2-³H] *n*-hexadecane was used as an internal standard. All other aqueous samples (skin rinsings, standards and washings) were monitored in a similar manner.

Faeces were freeze-dried and the sublimate was monitored for tritium. Aliquots of up to 350 mg of the residue were combusted in a Packard 305 sample oxidizer to determine the tritium content as tritiated water. Recoveries of greater than 98% were recorded from standards with counting efficiencies of up to 25%.

Full depth, 1 cm diameter punch autopsies of frozen skin were monitored for tritium either after combustion in a similar way to the faecal samples or after solubilizing in *Soluene* (Packard Instruments Ltd) at 40°C overnight and neutralizing with an excess of solid CO₂.

The protective patch was soaked in 50 ml ethanol at room temperature overnight and further extracted with 50 ml ethanol. The extracts were combined and aliquots were counted to determine the tritium content of the patch. Recovery of radio-activity by this method was better than 99%.

Blood tritium levels were determined by combusting up to 0.5 ml aliquots of blood in the Packard 305 sample oxidizer.

RESULTS

Turnover of subcutaneously injected Triclosan

The recoveries of tritium in the urine and faeces of female rats during 4 days are presented in *Table I*, from which it can be seen that 89.2% of the dose was recovered, some 33% being present in the urine. The faeces

Table I. Turnover of Triclosan injected subcutaneously

Time (days)	Tritium recovery ($\text{dpm} \times 10^{-6}$)		
	Urine	Faeces	Total
1	17.487 ± 5.079	23.210 ± 6.112	40.697 ± 8.722 (4)
2	3.589 ± 1.303	12.155 ± 4.011	15.744 ± 6.041 (3)
3	1.126 ± 0.840	2.289 ± 1.037	3.415 ± 1.149 (2)
4	0.857 ± 0.611	1.170 ± 0.610	2.027 ± 1.021 (2)
Total	23.059	38.824	61.883

Four female Colworth-Wistar rats (120 g) were injected with 0.5 ml of a 50% aqueous polyethylene glycol 400 solution containing 69.453×10^6 dpm [^3H] Triclosan. The rats were placed into individual metabolism cages and the excreta collected separately every 24 h for 4 days. The tritium content of the faeces was determined by combustion and scintillation counting and that of the urine by counting directly.

always contained the greater proportion of radio-activity throughout the 4 days. Using these data, a semi-logarithmic plot of total excreted tritium against time indicated a biological half-life of approximately 14 h. In the first two days 81.3% of the dose was excreted, and this figure is used to correct the recoveries in 2 days from rats treated topically with [^3H] Triclosan in shampoo or aerosol deodorant. The level of tritium in blood of subcutaneous-injected rats ranged from the equivalent of $0.2 \mu\text{g ml}^{-1}$ at 0.5 h to a maximum of $5.2 \mu\text{g ml}^{-1}$, at 6 h after injection and decreased over 24 and 48 h to approximately $0.3 \mu\text{g ml}^{-1}$ at 96 h.

Application of [^3H] Triclosan in shampoo

Some 85% of the [^3H] Triclosan applied to rat skin at various concentrations in the shampoo base was recovered in the rinse water and the patch, with additional smaller amounts remaining in the treated skin (*Table II*). The amount of [^3H] Triclosan residing in skin, at 48 h after the shampoo, was proportional to the concentration of [^3H] Triclosan in the shampoo applied to the skin.

The extent of penetration of [^3H] Triclosan through rat skin washed with shampoo was calculated from the amount of tritium in the excreta during the 2 days after treatment. Penetration of [^3H] Triclosan through rat skin increased in proportion to the concentration in the shampoo (*Table III*) but was independent of the duration of contact of the shampoo with the skin for periods up to 20 min before rinsing (*Table IV*). The concentration

Table II. Recovery of Triclosan from shampoo-treated rats

Application (%)	Application ($\mu\text{g}/7.5 \text{ cm}^2$)	Recovery at 48 h			Rats (No. of ♀)
		Rinsings (μg)	Patch (μg)	Skin ($\mu\text{g cm}^{-2}$)	
0.05	89 (15 cm^2)	76.5 ± 5.6	< 1.0	0.15	12
0.10	103	93.7 ± 1.7	2.8 ± 0.6	0.48	3
0.50	474	410 ± 63	7.4 ± 3.4	1.40	3
0.75	671	563 ± 38	6.6 ± 4.6	1.75	8
1.0	880	879 ± 51	18.6 ± 3.4	2.40	3
2.0	1664	1717 ± 65.6	26.9 ± 7.9	3.24	3

To 7.5 cm^2 (or 15 cm^2) clipped dorsal female rat skin, 0.1 ml (or 0.2 ml) shampoo containing various concentrations (w/v) of [^3H] Triclosan was applied followed by 0.1 ml (or 0.2 ml) H_2O and spread over the skin for 1 min. After a further 9 min the rats were rinsed, dried, a protective patch applied and transferred to individual metabolism cages. At 48 h after treatment the rats were killed and the patch and treated area of skin removed for analysis.

in blood, based on the radioactivity expressed as μg Triclosan, was very low and increased as the concentration of [^3H] Triclosan in the shampoo was raised (*Table III*), but it remained constant when the contact time was increased (*Table IV*). For a shampoo containing 0.05% [^3H] Triclosan the observed penetration was $0.16 \mu\text{g cm}^{-2}$ during a 48 h period. On adjustment for an 81.3% excretion of a subcutaneously-injected, known dose the corrected penetration figure is $0.197 \mu\text{g cm}^{-2}$. The total amount of [^3H] Triclosan penetrating through the treated area of skin is, therefore, 2.95 μg , or 3.3% of the applied amount. For the other concentrations, the extent of penetration ranged from 2.8% to 4.1%.

Application of [^3H] Triclosan in aerosol deodorant

The mean amount of spray used per application was $1.45 \pm 0.36 \text{ g}$ which at a concentration of [^3H] Triclosan of 0.1% meant that $1.45 \pm 0.36 \text{ mg}$ [^3H] Triclosan was sprayed at the shielded rats.

The mean concentration of [^3H] Triclosan in the $1 \times 15 \text{ cm}^2$ of shield around the treatment window was $13.2 \pm 6.2 \mu\text{g cm}^{-2}$ which was taken as a good measure of [^3H] Triclosan applied to the treated area of skin. At 48 h after treatment, the protective patch contained 41 μg [^3H] Triclosan and the residue in the treated area of skin was $9.7 \pm 4.8 \mu\text{g cm}^{-2}$. Recovery of tritium in the excreta was equivalent to $41.7 \pm 11.48 \mu\text{g}$ [^3H] Triclosan for six rats,

Table III. Effect of concentration on penetration of Triclosan from shampoo

Application (%)	Application ($\mu\text{g}/7.5 \text{ cm}^2$)	Blood ($\mu\text{g ml}^{-1}$)	Urine		Faeces		Total		Penetration ($\mu\text{g cm}^{-2}$)	Rats (No. of ♀)
			Urine	Faeces	Faeces	Total				
0.05	89 (15 cm^2)	0.044 \pm 0.003	0.98 \pm 0.19	1.42 \pm 0.71	2.40 \pm 0.50	0.16 \pm 0.06	12			
0.10	103	0.025 \pm 0.005	0.98 \pm 0.21	2.79 \pm 0.45	3.77 \pm 0.42	0.50 \pm 0.06	3			
0.50	474	0.038 \pm 0.070	2.85 \pm 1.62	8.40 \pm 2.26	11.25 \pm 0.89	1.50 \pm 0.12	3			
0.75*	695	0.047 \pm 0.011	6.79 \pm 2.40	10.41 \pm 3.23	17.20 \pm 0.38	2.30 \pm 0.35	8			
1.0	880	0.051 \pm 0.013	8.45 \pm 1.87	20.61 \pm 5.15	29.06 \pm 5.11	3.87 \pm 0.67	3			
2.0	1664	0.107 \pm 0.031	12.65 \pm 3.30	37.00 \pm 5.61	49.65 \pm 5.80	6.62 \pm 0.77	3			

To 7.5 cm^2 (or 15 cm^2) clipped dorsal female rat skin 0.1 ml (or 0.2 ml) shampoo containing various concentrations (w/v) of ^3H Triclosan was applied followed by 0.1 ml (or 0.2 ml) H_2O and spread over the skin area for 1 min. After a further 9 min the rats were rinsed, dried and transferred to individual metabolism cages. Urine and faeces were collected separately every 24 h for 2 days when the rats were killed and blood radioactivity measured.

* Mean data from Table IV.

Table IV. Effect of contact time on penetration of Triclosan from shampoo

Duration (min)	Rinsings (μg)	Patch (μg)	Skin residue ($\mu\text{g cm}^{-2}$)	Blood $\mu\text{g ml}^{-1}$	Total		Penetration ($\mu\text{g cm}^{-2}$)	
					Urine	Faeces		
1	481	9.9	1.84	0.047	8.54	8.18	16.72	2.23
5	575	6.5	1.75	0.057	7.53	10.70	18.23	2.43
10	561	5.5	1.95	0.043	6.12	12.60	18.72	2.50
20	583	4.6	1.43	0.042	4.95	10.17	15.12	2.02

To 7.5 cm^2 clipped dorsal female rat skin 0.1 ml shampoo containing 0.75% (w/v) ^3H Triclosan (671 μg) was applied with 0.1 ml H_2O . After 1, 5, 10 or 20 min contact, the skins were rinsed and dried and the rats transferred to individual metabolism cages. The excreta were collected separately every 24 h for 2 days when the rats were killed. Samples were taken for determination of radioactivity as in methods section. Results are mean of two animals.

thus the observed penetration was equivalent to $5.57 \mu\text{g cm}^{-2}$. Adjustment for an 81.3% excretion of a known dose given subcutaneously, gives a corrected penetration figure of $6.85 \mu\text{g cm}^{-2}$.

Application of [^3H] Triclosan in ethanol

It was difficult to spray a standard dose of aerosol deodorant on to rat skin. Thus, ethanol, which is one of the main ingredients of the aerosol formulation, was used as a solvent in which to apply a more accurately known amount of [^3H] Triclosan to experimental animals. The results of the penetration studies from ethanol are recorded in *Tables V and VI*.

Of the [^3H] Triclosan applied to skin, a high proportion was recovered from the patch lint or in the treated skin area. It can be seen (*Table V*) that in some instances the treated skin contains the larger amount of [^3H] Triclosan, while in other cases the patch lint contains the greater proportion. The level of tritium in the blood calculated as ppm Triclosan (i.e. $\mu\text{g ml}^{-1}$) rises to a maximum at about 6 h after application and falls steadily thereafter.

Based on the recovery of tritium in the urine and faeces during the first 48 h of the experiment (*Table VI*) the equivalent of $36.3 \pm 5.88 \mu\text{g}$ [^3H]

Table V. Penetration of Triclosan from ethanol

Rat	Time after application (h)	Residue (μg) in		Tritium in blood (as ppm Triclosan)
		Patch	Skin (7.5 cm^2)	
1	1	21.06	163.63	0.294
2		138.98	27.56	0.070
3	2	14.73	166.73	0.194
4		21.04	167.50	0.362
5	4	128.16	27.65	0.114
6		133.67	23.85	0.190
7	6	93.08	38.48	0.301
8		112.32	29.96	0.228
9	24	81.26	25.75	0.241
10	48	79.36	21.84	0.119
11	96	69.84	17.74	0.072
12		63.43	16.33	0.074

To 7.5 cm^2 clipped dorsal female rat skin was applied 0.2 ml ethanol containing 162 μg [^3H] Triclosan. The alcohol was allowed to dry and the skin protected with a non-occlusive patch. The rats were placed in individual metabolism cages, killed and the patch, treated skin and blood, urine and faeces were collected separately every 24 h and analysed for tritium.

Table VI. Excretion of tritium from rats treated with [³H] Triclosan in ethanol

Time (days)	Tritium recovery (dpm × 10 ⁻⁶)		
	Urine	Faeces	Total
1	0.702 ± 0.216	1.233 ± 0.353	1.935 ± 0.240 (4)
2	0.406 ± 0.095	1.277 ± 0.481	1.683 ± 0.410 (3)
3	0.234 ± 0.064	0.437 ± 0.153	0.671 ± 0.089 (2)
4	0.197 ± 0.016	0.189 ± 0.047	0.386 ± 0.062 (2)
Total	1.539	3.136	4.675

Four female Colworth-Wistar rats (120 g) were treated topically with 0.2 ml ethanol containing 16.09×10^6 dpm [³H] Triclosan over 7.5 cm² clipped dorsal skin. Non-occlusive patches were fixed in position and the rats placed into individual metabolism cages. The excreta were collected separately every 24 h for 4 days. The tritium content of the faeces was determined by combustion and scintillation counting and that of the urine by counting directly.

Triclosan were recovered. This gives an observed penetration of 4.84 µg [³H] Triclosan per cm² skin, which on adjustment for an 81.3% excretion of a subcutaneous dose gives a corrected penetration of 5.96 µg cm⁻². The total amount of [³H] Triclosan penetrating through the treated area of skin is therefore 44.68 µg or 27.6% of the applied amount.

DISCUSSION

In the present report, some 33% of the dose given subcutaneously to female rats was recovered in the urine during 96 h. The difference from a previous study (5) in which only 8% was excreted in the urine of male rats injected intraperitoneally with [³H] Triclosan was shown by subsequent experiments (unpublished data) to be due to sex and not to the route of administration.

The half life of both intraperitoneally and subcutaneously-injected [³H] Triclosan was approximately 14 h whereas after topical application some 23 h were required to excrete 50% of the absorbed dose, an increase which reflects the reservoir effect of the stratum corneum (6). The fact that there may be such a reservoir is supported by the finding that the same amount of [³H] Triclosan is absorbed through skin despite an increase in the duration of contact with the skin of the shampoo.

The method of application of the shampoo and deodorant was selected to reproduce the way in which the consumer would use these types of

products. Thus the shampoo was applied to the skin, diluted with water and the excess, after varying periods of contact, was rinsed away. The aerosol on the other hand was applied and allowed to dry on the skin. The penetration of [^3H] Triclosan from the shampoo was $0.197 \mu\text{g cm}^{-2}$ and was $6.85 \mu\text{g cm}^{-2}$ from the aerosol, or some thirty times more in favour of the aerosol at these particular product concentrations of [^3H] Triclosan. Even at comparable concentrations of [^3H] Triclosan (i.e. 0.1% (w/v)) the penetration is still some eleven times greater from the aerosol. Thus, the composition and mode of use of different products containing [^3H] Triclosan is very important in determining the extent of penetration of the germicide.

Based on the work described by Feldman and Maibach (7), Maibach *et al.* (8) and Bartek, Labudde and Maibach (9) together with the data collated by Tregear (10), we consider that the permeability of rat skin may be similar to that of human scalp and axilla. Using the experimental data obtained from the present rat experiments together with a no-effect level from a 3-week target organ test in the rat of $200 \text{ mg kg}^{-1} \text{ day}^{-1}$ (unpublished observations) we can make calculations of the safety-in-use of Triclosan for the average woman of 55 kg body weight. Assuming the area of the scalp and hands is 1350 cm^2 , then for a penetration of $0.197 \mu\text{g cm}^{-2}$, the absorbed dose is $4.8 \mu\text{g kg}^{-1}$, which is 42 000 times less than the no-effect level observed in the target organ test. The highest concentration of [^3H] Triclosan gave a minimum of 1000 times less than the no-effect level.

From the aerosol deodorant if the spray is used twice daily for 2 s on each axilla of 50 cm^2 , then the absorbed dose, at $6.85 \mu\text{g cm}^{-2}$, is $24.9 \mu\text{g kg}^{-1}$ which is 8000 times less than the no-effect level in the target organ test.

Thus, we conclude on the basis of the percutaneous absorption and toxicity data available, that extremely small proportions of the no-effect level of Triclosan are likely to be absorbed through adult human skin treated with shampoo containing 0.05% (w/v) Triclosan or with an aerosol deodorant containing 0.1% (w/v) Triclosan.

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