In Vitro Test of Nicotine's Permeability through Human Skin. Risk Evaluation and Safety Aspects

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Permeability tests with Franz' diffusion cells and an *in vitro* test model were made to evaluate the importance of dermal absorption of nicotine as a pathway for intoxication. Studies were carried out to ensure that safety procedures, when spilling nicotine on skin, are sufficient to prevent poisoning. Pure nicotine and nicotine in various concentrations in water or ethanol were applied on human skin or gloves in Franz' cells. Washing was simulated by removing nicotine from skin after 3 or 5 min.

Permeation rate (flux) and lag time were calculated and estimated for human skin. Different glove materials were tested for their nicotine breakthrough time. Flux depended on concentration in a non-linear way when nicotine–water solutions were tested. Highest flux was found in 50% w/w nicotine dissolved in water. Solutions with low concentration of nicotine (1% w/w) dissolved in water had a similar permeation rate to 100% nicotine. Flux was found to be low when using ethanol as a vehicle; flux was also pH-dependent. The nicotine–water solution containing acetic acid had the lowest flux.

The tests where nicotine was washed away revealed that skin served as a possible nicotine depot, because nicotine concentration in the receptor compartment continued to increase after removing the nicotine from the surface. The length of contact time affected the amount of substance passing the skin, resulting in great difference between 3 and 5 min contact time, 5 min giving higher nicotine concentration and 3 min lower. This emphasizes the importance of washing away nicotine spilled on skin rapidly. Two glove types were tested and they were found to be appropriate in their use with nicotine if changed regularly. © 1999 British Occupational Hygiene Society. Published by Elsevier Science Ltd. All rights reserved.

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INTRODUCTION

Penetration through skin and subsequent systemic absorption is the most significant extrapulmonary route of uptake in occupational exposure to chemicals, and that uptake is impossible to assess with conventional ambient air monitoring methods. Acute toxicity and long term hazards have ensued from skin contamination by dust and liquids of toxic and carcinogenic substances that are effective penetrants (Ness, 1994).

Nicotine's high solubility in both polar and non-polar solvents (log $Kw = 1.17$) and its low molecular weight (162.2 g/mol) makes it a theoretically efficient penetrant. This, combined with its highly acute toxic

effect on the body and low deadly dose (0.5–1 mg/kg, LD_{50} or 30–60 mg for an adult human) has, in some cases, resulted in severe poisoning from percutaneous absorption (Simpson and Curtis, 1974; Gosselin *et al.*, 1984). As early as in the 1930s, Faulkner (1933) showed that the dermal route is a possible one for nicotine intoxication, provided that nicotine is in its base form. Cases have been reported where skin contact with nicotine base solution lead to vomiting, illness, and other symptoms on serious poisoning (Benowitz *et al.*, 1987). Other investigations also showed that nicotine had a high potential to enter the organism dermally (Travell, 1960; Gehlbach *et al.*, 1979). Considering how toxic nicotine is, it is understandable that rigorous control and precautions are taken when handling nicotine.

The skin offers excellent protection against many harmful agents. According to Scheuplein and Blank (1971), the main diffusion barrier is the stratum corneum, and nicotine's capability to penetrate human

skin depends on the way it passes through this barrier. As seen in Fig. 1 the stratum corneum is the outermost layer in the epidermis. It is composed of dead, keratinized and desiccated corneocytes packed very closely and surrounded by a lipid mixture of phospholipids, sphingolipids and neutral lipids (Elias *et al.*, 1981). It has been compared with the way brick and mortar relate to each other, where the brick represents the corneocytes and mortar the lipid phase (Michaels *et al.*, 1975). This model simplifies what in fact is a very complex structure. The lipids among the corneocytes arrange in lamellae and this is one of several reasons that makes the skin to a powerful barrier (Forslind *et al.*, 1998). The stratum corneum's construction is ideal for maintaining milieus in the body free from foreign substances. It also keeps homeostasis in the body. It effectively gives protection against polar as well as nonpolar substances. But substances that are soluble in both polar and nonpolar solvents permeate skin more easily (Oakley and Swarbrick, 1987). Nicotine is that kind of a substance. The molecule is also compact, thus eliminating steric hindrance when entering.

There are several factors affecting the rate at which chemicals permeate human skin, and among them are wounds, body site, age and sex (Ness, 1994). All these factors cannot be taken into account in a model because of the complexity which would then arise. A simple model, which makes it possible to study the percutaneous penetration of chemicals, is the Franz' diffusion cell. It gives a good approximation of the skin permeation rates of different substances in various membranes.

The diffusion is passive when neutral molecules permeate through skin, and the rate of the substance diffusion ($\mathrm{d}Q/\mathrm{d}t$) can be described by the following relationship:

$$\frac{\mathrm{d}Q}{\mathrm{d}t} = P_S(C_D - C_R), \qquad (1)$$

where P_S is the skin permeability coefficient and C_D and C_R are the substance concentrations in the donor (D) and the receptor (R) medium of the Franz' cell. P_S is constant under fixed conditions.

The skin permeability coefficient depends upon the following terms:

$$P_S = \frac{K_S D_{SS}}{h_S}. \qquad (2)$$

K_S is the partition coefficient for the penetrant between the delivery system and the skin, D_{SS} is the diffusion of the penetrant at steady state, and h_S is the thickness of the skin.

Integration of Eq. (1) gives

$$Q = P_S(C_D - C_R)t. \qquad (3)$$

The flux is the slope of cumulative uptake of substance through a unit of surface area of skin (Q in μg/cm^2) as a function of time.

It is very important to prevent dermal uptake of nicotine. This is achieved with good protective clothing and gloves. Unfortunately, glove suppliers mostly do not give permeation data such as breakthrough time and permeation rate for less com-
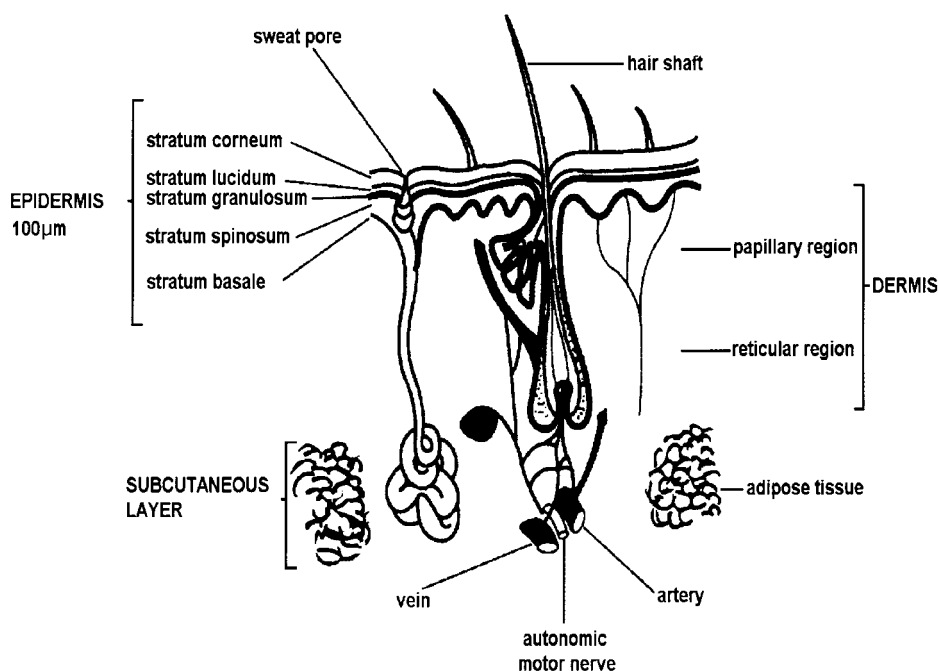

Fig. 1. Skin structure.

monly used agents such as nicotine. The only exception is the 4H glove (4H, Safety 4 Inc.). This glove protects against nicotine for up to four hours, but is too clumsy for refined work. The lack of nicotine permeation data for other gloves used in Pharmacia & Upjohn when handling nicotine in the pharmaceutical production and in the laboratories, made acquisition of permeation data necessary. The gloves used, other than the 4H glove, are a double layer of N–DEX (Best Company) in pharmaceutical production and Touch N Tuff (Ansell Edmont Company) used by the laboratory personnel.

OBJECTIVE

The following questions were raised:

- How long does it take for nicotine to penetrate the skin? The lag time is used to estimate this time.
- Does the skin serve as a possible nicotine reservoir even after the skin is thoroughly rinsed with recommended solutions? The skin's ability to deposit nicotine is assessed with the rinsing tests.
- How do lag time and permeation rates change for different concentrations of nicotine in water and ethanol?
- To ensure that safe conditions prevail when nicotine is handled in production and in the laboratories, two types of gloves were tested for their nicotine breakthrough time.

MATERIALS AND METHODS

Materials

Methanol, LAB–SCAN; ethanol, Kemetyl; acetic acid, glacial, Merck, pro analysi; sodium hydrogen phosphate, Merck, pro analysi; potassium di hydrogen phosphate, Merck, pro analysi; nicotine, batch No.: 9702I103, Pharmacia & Upjohn; fluent soap, Marlén, Nordex Company.

The Franz' diffusion cell

Franz' diffusion cells were obtained from the Crown Glass Company.

A cell is shown in Fig. 2. This is a static, one-chambered diffusion cell. The substance is applied on the membrane (skin or glove) through the open cell-cap. The receptor volume is 6 or 7 ml. A stirring bar maintains homogenous temperature and concentration in the cell body. Heated water circulates through glass manifolds and the water jacket to control temperature. The temperature in the saline solution is 37°C.
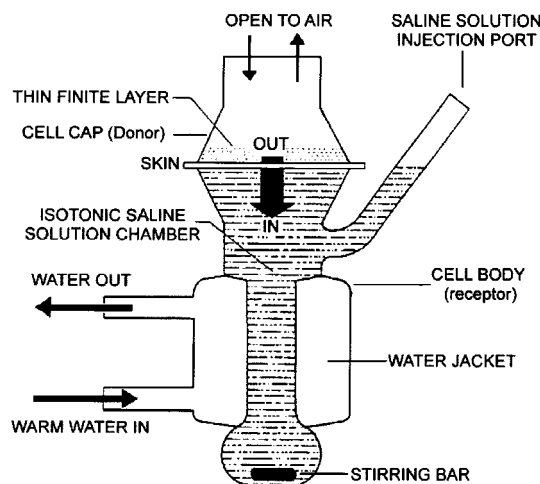

Fig. 2. Franz' diffusion cell.

Membranes

Nitrile glove, N–DEX, Best Company, thickness: 0.114 mm; Nitrile glove, Touch N Tuff, Ansell Edmont Company, thickness: 0.100 mm; Human skin, plastic-surgery waste products, collected from plastic surgery clinic by Pharmacia & Upjohn, Hilleröd, Denmark.

Skin preparation

Human skin was obtained from plastic-surgical operations. The skin originated from two body sites, either the mammae or the abdomen and the donors were women; the donor's age was known in some cases. For each nicotine solution tested, 2 donors were used and at least 6 replicates (3 pieces of skin × 2 donors), of the Franz' cell tests were made for each solution. In total, skin from 17 donors was used. The remaining skin from each donor was frozen in order to repeat tests when needed. The skin was pinned to a plastic cutting board with bending foam rubber beneath and then prepared with dermatome at a thickness setting of 408 μm (position 16). Figure 1 illustrates the parts of the skin included at this thickness. Careful examination of the skin, by holding it to a light source, was done to establish that it was free from scars, other unevennesses and dried up parts. Then the skin was frozen until tested. Freezing the skin does not influence the permeability, according to Bronaugh *et al.* (1986). The skin was allowed to thaw for one hour before the experiment (note should be taken that the skin slices were thin and almost free from subcutaneous fat, i.e. the thawing time was short).

Glove preparation

Parts of the gloves from Touch N Tuff, Ansell Edmont Company and N–DEX, Best Company were cut out and mounted in the Franz' cell for breakthrough time studies.

Table 1. Prepared nicotine solutions

Nicotine solutions	Concentration of nicotine (% w/w)	Concentration of nicotine (g/l)	Other components	pH in solutions
Solution I	1	10.5	Water, phosphate buffer	7
Solution II	10	104.5	Water	10.9
Solution III	20	204.6	Water	11.1
Solution IV	50	502.6	Water	11.2
Nicotine	100	—	—	—
Solution V	20	202.8	Water, acetic acid	4.6
Solution VI	8	64.0	Ethanol	—
Solution VII	20	165.1	Ethanol	—

Preparation of the nicotine solutions

Concentrations and formulae used in the *in vitro* tests are given in Table 1.

Nicotine HPLC analysis method

Analysis was performed on the 100 μl samples withdrawn from the receptor compartment through the injection port of the cell. Injector, Autosampler Waters 717+. Analytical column, Hypersil Hypurity Elite C18, 5 μm, 100 × 4.6 mm, part No. 22105-064. Mobile phase, 0.08 M phosphate buffer pH 6.5/methanol/trietylamine (55.8/40/4.2). Flow rate, 1 ml/min. Detection, Variable Absorbance detector Waters 486 at 254 nm. Pump, Waters 600E. Standards, pure nicotine dissolved in water and diluted to cover the interval 0.5 μg/ml–500 μg/ml. Injected volume, 10 μl.

Experimental procedure

The skin was thawed for one hour and then cut into pieces that fitted and covered the O-ring. The two compartments were sealed together with a clamp. Phosphate buffer pH 7.0, 0.02 M was pipetted into the injection port and all bubbles in the receptor compartment were eliminated. A stirring bar magnet was added. The cell was allowed one hour to come to uniform temperature. The test solution, 360 μl, was applied on the skin, but only 100 μl was applied when testing pure nicotine because of the high concentration. With 360 μl test solution, a 2 mm thick layer was obtained since the skin area exposed was 1.8 cm². Samples (100 μl) were withdrawn and replaced with fresh buffer via the injector port at intervals, 0, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25, 30, 40, 50, 60 and 80 min. For this purpose, a Finnpipette with extended tip was used. Each nicotine solution was tested in 6 replicate cells. Samples were assayed by HPLC for nicotine concentrations immediately; otherwise samples were stored in a refrigerator. The results that gave rise to extremely high flux values, were classified as leakage of nicotine through defect skin. In those cases, the test was discarded and repeated with new skin.

The rinsing test with 50% w/w nicotine water solution was performed somewhat differently. The solution was removed after 3 or 5 min from the donor compartment and the skin was rinsed with 4 portions × 2.5 ml 2% acetic acid. Rinse of 100% nicotine from skin was also performed after 5 min with 4 portions × 2.5 ml 1% soap solution. The samples were then withdrawn according to the intervals given above.

Gloves were subjected to the same conditions as skin during the test, except that the sample withdrawing times differed. The tests were interrupted earlier because of minor interest in permeation rates. The only solution tested on glove was pure nicotine.

The flux Eq. (3) was determined separately for each cell used, and a mean flux for the six cells was also calculated. The lag time, which is the elapsed time between nicotine contact with skin and detection in the receptor compartment, was estimated from the flux profile graphs (e.g. Figure 6). The lag time was taken into account when calculating flux.

The glove breakthrough time was estimated in the same way as lag time.

RESULTS

Flux and lag time for different solutions on skin

Mean flux and lag time for different solutions applied on skin are shown in Table 2. The interval of the lag time spreading is also given.

Flux clearly depends on concentration of nicotine but not linearly. The relationship is presented in Fig. 3. Mechanisms involved will be further discussed. The fluxes are mean values of 6 cells (3 cells ×2 donators).

The difference between ethanol and water as vehicles is evident. Flux is remarkably low for nicotine dissolved in ethanol. The acidic 20% w/w nicotine solution gave the lowest flux.

Lag times were evaluated for every single cell and a mean of 6 cells (exceptions were made in those cases where a leakage was detected) was calculated.

Donor dependency

Study of the flux curves in order to find donor-grouping tendencies was fruitful is some cases, as seen in Fig. 6. Discrepancies from this were also found.

Table 2. The calculated fluxes and evaluated lag times with ranges

Nicotine solutions applied on skin, conc. in %w/w	Flux (μg/cm^2×h)	P_S (cm/h×10^3)	Mean lag time (min)	Lag time range (min)
1% in water, phosphate buffer	88.2	8.4	10.0	7.5–18
10% in water	880.9	8.4	7.5	5–10
20% in water	883.4	4.3	8.8	7.5–10
50% in water	1341.5	2.7	9.5	7.5–10
100% nicotine	82.0	0.08	13.0	5–20
8% in ethanol	6.1	0.095	10.0	7.5–12.5
20% in ethanol	12.3	0.075	10.0	5–15
20% in water, with acetic acid (pH = 4.6)	3.4	0.017	10.0	10
50% in water, rinsing with 2% acetic acid after 3 min	—	—	8.0	7.5–10
50% in water, rinsing with 2% acetic acid after 5 min	—	—	8.3	7.5–10
100% rinsing with 1% soap solution after 5 min	—	—	23.8	20–27.5

Table 3. Breakthrough time for gloves

Glove	Breakthrough time (min)	Breakthrough time ranges (min)
Touch N Tuff	13	12–15
N-DEX, 1 layer	7.5	6–9
N-DEX, 2 layers	38	30–42

Rinsing

After application of 50% w/w nicotine water solution and allowing a 3 or 5 min contact time, rinsing of skin with 2% acetic acid from the donor compartment side was made to see how effectively it could be carried out. Despite almost all nicotine being removed from skin, concentrations in receptor chamber continued to rise (Fig. 4). The difference in the amount of permeated nicotine between 3 and 5 min contact time is great and 5 min gives higher values. When pure nicotine was removed with a soap solution after 5 min, the lag time increased.

The amount of nicotine that reached the receptor was somewhat higher than for the 3 min contact time with 50% w/w nicotine in water solution.

Glove tests

Breakthrough time was sought in the investigation of one and two layers of N–DEX (Best Company) and one layer of Touch N Tuff, (Ansell Edmont Company). The breakthrough time more than doubled for two layers of N–DEX, compared to one layer (Table 3). One layer of Touch N Tuff was
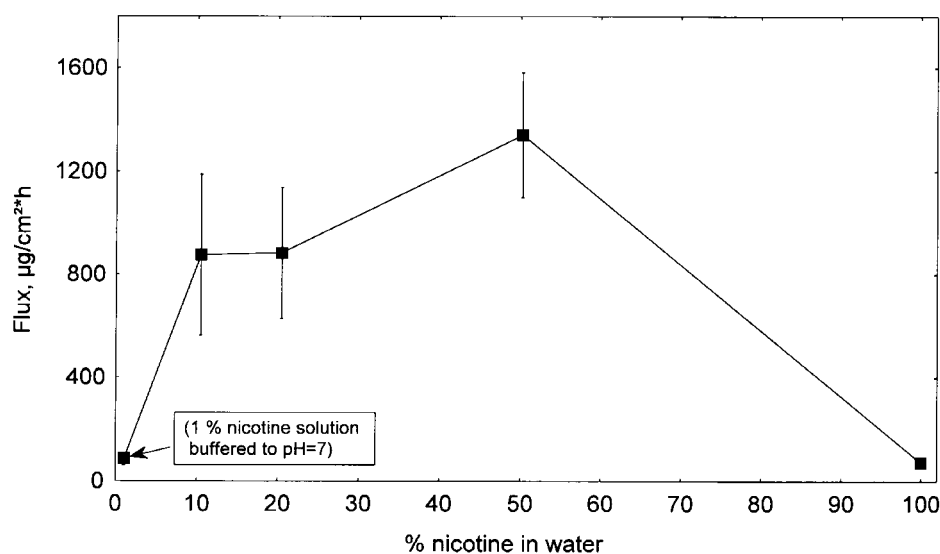

Fig. 3. Flux plotted against percent nicotine in water applied on skin.
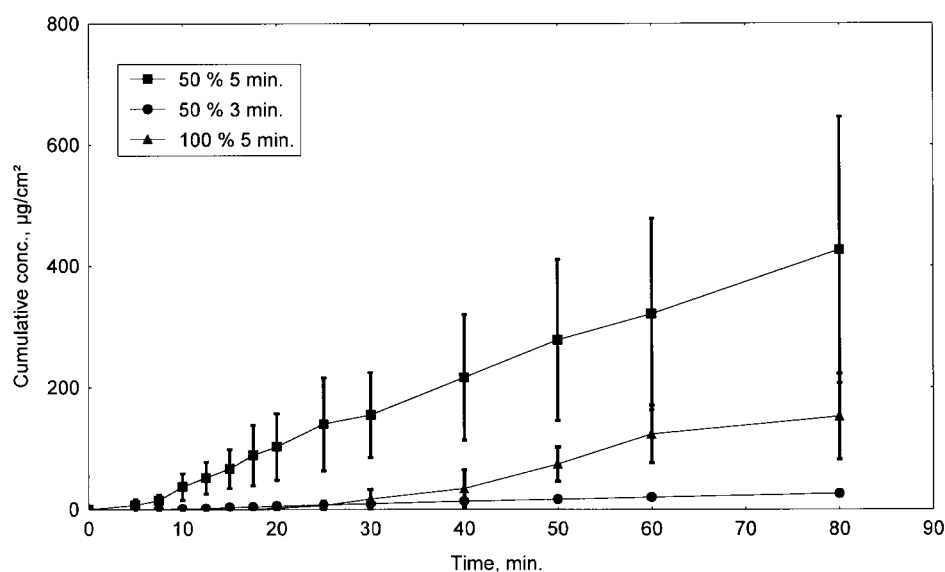
Fig. 4. Flux profiles for 50% w/w nicotine in water, rinsed off after 3 and 5 min with 2% acetic acid and 100% nicotine washed off after 5 min with 1% soap solution.

more resistant than one layer of N–DEX and the breakthrough time was a few minutes longer for Touch N Tuff.

DISCUSSION

It was unexpected that the rate at which pure nicotine and nicotine in ethanol permeate skin is much lower than it is for nicotine–water solutions, as shown in Table 2. The permeation rate for different concentrations of nicotine in water is shown in Fig. 3. There is a maximum at 50%.

Why do pure nicotine and nicotine in ethanol have lower permeation rates than nicotine in water and why is the permeation rate highest for the 50% w/w solution of nicotine in water? According to Fick's law, a stronger concentration should give a higher permeation rate.

Several factors influence on the permeation rate, e.g.:

- The partition coefficient: nicotine/water, nicotine/ethanol, nicotine/lipids, nicotine/phosphate buffer;
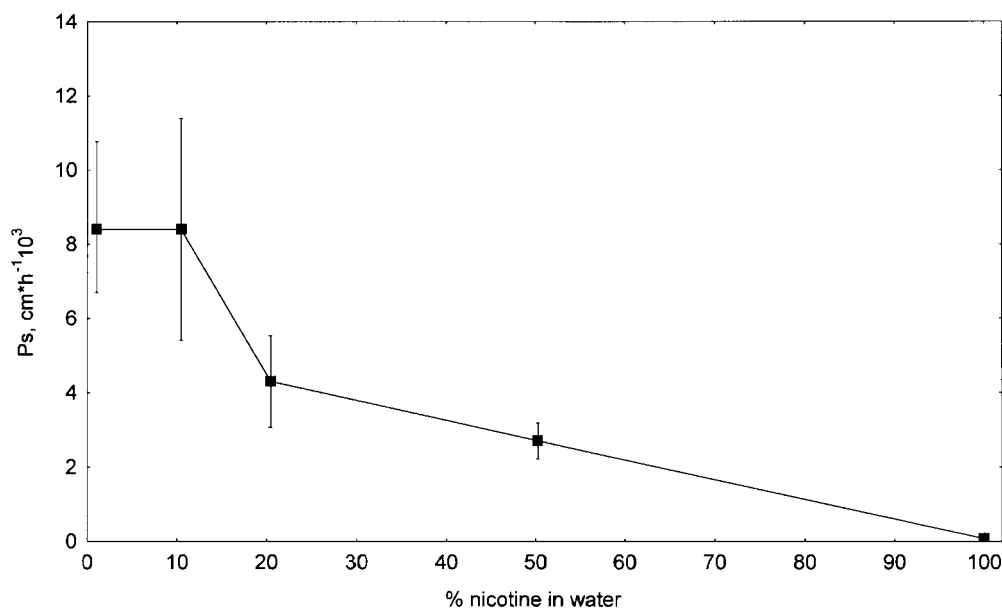- Concentration gradient.

Fig. 5. Ps. skin permeability coefficient as a function of nicotine water concentration.

Table 4. Skin area exposed to reach 500 μg/h

Nicotine solutions, conc. (% w/w)	Area (cm^2) needed for an uptake of 500 μg/h
1% in water, phosphate buffer	5.7
10% in water	0.6
20% in water	0.6
50% in water	0.4
100% nicotine	6.0
8% in ethanol	82.0
20% in ethanol	40.8
20% in water, acetic acid (pH = 4.6)	147.0

The permeability coefficient (P_S) curve (Fig. 5) drops at higher concentrations and this gives us reason to believe that a possible explanation of the permeability rate maximum for nicotine solutions in water is the partition coefficient theory. When nicotine is solved in water, the partition coefficient skin/vehicle increases because nicotine prefers the fatty surroundings in the skin. The driving force which facilitates the permeation of nicotine is enhanced by dissolving it in water. This is clear from Fig. 3. When nicotine concentration rises over 50% w/w, the nicotine has a higher affinity to itself than to the system of lipids and water, and the skin/vehicle partition coefficient decreases, resulting in a lower permeation rate at higher concentrations (Walters, 1986). When reviewing the literature, another possible theory found for this behaviour of the flux is that the barrier properties of the skin are impaired by the hydration of the stratum corneum when water is present. The barrier would then be improved by dehydration of the stratum corneum when exposed to pure solvent (Johansson and Fernström, 1988).

A similar relationship between permeation rate and concentration of nicotine in water has been obtained in other studies and even for other substances, with declining rate when concentration of the substance gets stronger (Oakley, 1986; Johansson and Fernström, 1988). The permeation rate for nicotine dissolved in ethanol is very low compared to nicotine in water. This illustrates that the vehicle has a great influence on the flux. If nicotine has a higher affinity to ethanol than to lipids and phosphate buffer, the driving force will be low and nicotine will stay in the upper compartment.

The lag times for all tested solutions are not easily distinguishable.

It is also of great interest to evaluate if nicotine is deposited in skin. This was tested by quantitative removal of nicotine from the donator compartment. Figure 4 shows the flux profiles for 50% w/w nicotine in water when the solution is removed from the receptor compartment after 3 or 5 min and the skin is rinsed with 2% acetic acid. It also shows the flux profile for 100% nicotine rinsed off with 1% soap solution after 5 min. There is a big difference in the total amount of permeated nicotine, which illustrates how important it is to clean the skin rather soon after contamination.
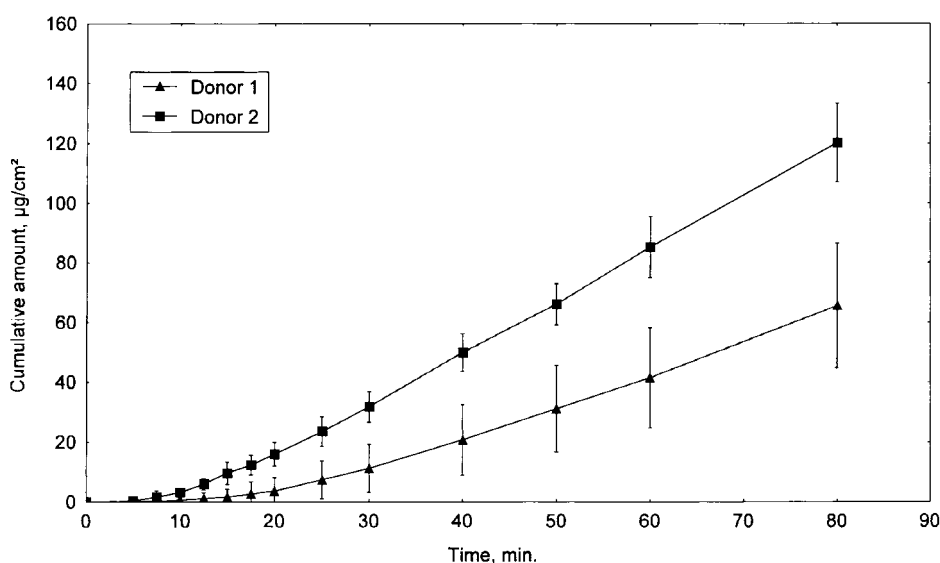

Fig. 6. Flux profile for 1% w/w nicotine dissolved in water (buffered to pH 7), when applied on two different donors.

Table 5. Area needed to achieve 30 mg with 15 min contact time

Nicotine solutions, conc. (% w/w)	Contact area (dm²) needed for an uptake of 30 mg during 15 min	
1% in water, phosphate buffer	13.6	
10% in water	1.36	
20% in water	1.36	
50% in water	0.9	(half palm)
100% nicotine	14.63	(an arm)
8% in ethanol	197	
20% in ethanol	98	
20% in water, acetic acid (pH = 4.6)	352	

With 100% nicotine rinsed off after 5 min with a soap solution, the lag time is long, but a rather large amount still enters the receptor compartment. To sum up the rinsing tests, it is established that if contact time is prolonged because of unawareness of nicotine contamination on skin, even rinsing could be insufficient to prevent poisoning because the skin itself would act as a reservoir for nicotine. As a consequence of this, it is important to rinse off nicotine spilled on skin as soon as possible to preclude an accumulation of nicotine in skin, and it is also important to protect hands to eliminate contact with nicotine.

The use of protective equipment when handling nicotine is necessary. If gloves are contaminated with nicotine, the amount of nicotine that reaches the skin depends on how long the gloves are used. The gloves must be changed within the breakthrough time. The breakthrough time for pure nicotine on gloves is shown in Table 3. Double layer N–DEX gloves give a good protection for at least 20 min, which is the routine for changing gloves in Pharmacia & Upjohn's pharmaceutical production. The staff in the laboratories use Touch N Tuff. This glove gives a good protection for approximately 10 min. If gloves are not changed regularly, an occlusion situation could arise and initiate a fast uptake of nicotine, as a result of the humid conditions between skin and glove.

The variation in flux for every solution, given as standard deviation in all the graphs, depends on the donor and the condition of the skin. This is not unexpected because of the variation in biological membranes. Factors influencing the permeation rate are biological, and biological material is very complex and heterogeneous. The results will vary in agreement with this, and the flux profiles for different donors which are shown in Fig. 6 prove this. The intra individual variation is small compared to the variation between different donors.

The result from this study made it possible to compare nicotine dermal exposure with the airborne exposure and also the exposure of nicotine from smoking and the use of nicotine patches. The Occupational Exposure Limit (OEL) for nicotine is 500 $\mu g/m^3$ air in many countries, e.g. in USA (American Conference of Governmental Industrial Hygienists, 1998). During an easy-to-moderate work loading, a human adult breathes about 1 m^3/hour. The size of contaminated skin area needed to achieve the same uptake (500 μg) as the one-hour exposure in the OEL-concentration is shown in Table 4. When studying these values, it might be concluded that enough pure nicotine to cover 6.0 cm^2 of skin is not spilled easily and the risk might be underestimated. These values, however, should be treated critically since they only reflect the skin samples' condition. In reality, the skin might be wounded, or nicotine could be diluted with perspired moisture, and this would give rise to higher uptake. Nicotine is a compound with high acute toxicity and should be treated as such.

The contact area with each solution needed to achieve the deadly dose of 30 mg nicotine (assuming contact time is 15 min) is given in Table 5. The average lag time, 10 min, is excluded. The area of a palm is 2 dm^2 and an average surface area for the whole body is 2 m^2.

It is very important that nicotine is handled with respect and that all precautions are followed. This give safer working conditions and minimizes the risk of poisoning. All personnel that handle nicotine should be very aware of the risk of nicotine and should be given information and safety instructions. It is also important that the dermal pathway is not neglected. Neglecting nicotine's skin penetrative properties could be fatal.

CONCLUSIONS

Flux was found to depend on the concentration in a non-linear fashion. The highest flux was found in the 50% w/w nicotine–water solution and 100% nicotine had similar flux as 1% w/w nicotine–water solution. The lowest flux was found in the acidic nicotine solution. Nicotine in ethanol gave also a very low flux.

Although rinsing of nicotine from the donor compartment was performed, levels of nicotine in the receptor compartment continued to increase. This showed that if contact time was long enough to achieve absorption, skin would function as depot for nicotine and rinsing would not draw it out. For that reason, careful washing as soon as possible when spilling nicotine on skin is very important.

Glove N–DEX and Touch N Tuff was found to be appropriate for use when handling nicotine. A single layer of Touch N Tuff had a longer breakthrough time than N–DEX.

Pedersen, Pharmacia & Upjohn, Hilleröd, Denmark, for provided skin.

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