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Influence of Evaporation and Solvent Mixtures on the Absorption of Toluene And *n*-butanol in Human Skin in Vitro

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The influence of forced ventilation on the percutaneous absorption of butanol and toluene was studied in vitro. Human skin was exposed to the neat solvents and the solvents in binary mixtures with each other and in ternary mixtures with chloroform:methanol. The exposure was either unventilated or ventilated with various flow rates. At the ventilated exposure the skin absorption of all solvents and solvent mixtures was markedly reduced compared to unventilated exposure. Exposure with solvent mixtures increased the amounts of solvent absorbed as well as absorption rates. The absorption of the butanol component was most influenced. Increase in absorption was 11 to 9 times depending on whether toluene or chloroform/methanol was cosolvent. There was also an interindividual variation of absorption rate, varying with a factor of 3.5 for toluene and 4.3 for *n*-butanol within the 3 skin donors used.

Skin absorption of volatile organic solvents at continuous ventilated conditions is related to their volatility and to the ventilation rate.

A sufficient workplace ventilation is an important occupational hygienic measure not only to reduce exposure via respiration but to reduce absorption via the skin of volatile compounds as well.

Keywords: n-butanol; toluene; skin absorption; ventilation; solvent mixtures

INTRODUCTION

Several compounds the skin encounters in the occupational or leisure time environment have a relatively high vapour pressure and are hence lost from the skin surface not just by absorption but also by evaporation (Spencer *et al.*, 1979; Reifenrath and Robinson, 1982; Hawkins and Reifenrath, 1984, 1986; Reifenrath and Spencer, 1989; Wester *et al.*, 1992). Some volatile chemicals are placed on the skin intentionally to hide smell, attract or repel representatives of the same or other species, for example, deodorants, perfumes and mosquito repellents, respectively. Skin exposure is, on the other hand, unintentional to numerous chemicals; these may be absorbed in quantity and exert mild to severe local or systemic effects for example, as with the organic solvents. The organic solvents constitute a heterogeneous group of structurally and physicochemically variable chemical compounds where the variation lies in volatility, molecular weight, lipophilicity, water solubility and other properties. However, they have several properties in common that make them useful in a vast number of industrial applications: fluidity, inexpensiveness and high lipid solubility. The use of organic solvents worldwide is large and they are applied in numerous applications for example, solvents for organic compounds, intermediates in organic synthesis, bases for glues, paints, degreasing and cleansing agents. The handling of organic solvents and solvent containing products often involves direct skin contact either by accident or by neglect to wear sufficient skin protection and skin absorption is well documented. Organic solvents are generally toxic when absorbed systemically; the most common toxic effect is inducing narcosis. Experimental data show that several of the solvents may also be absorbed in sufficient quantities to be lethal when

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the skin is exposed (Wahlberg and Boman, 1979). Most organic solvents also induce local toxic reactions following their skin exposure including necrosis, erythema, and edema (Kronevi *et al*., 1979, 1981; Wahlberg, 1984a,b, 1989). A few solvents or their metabolites are contact sensitizers (Fregert *et al*., 1963; Pirilä and Siltanen, 1958; Sjöborg *et al*., 1984; Karlberg *et al*., 1991). At unoccluded skin exposure to solvents the major route of loss of substance from the surface is through evaporation, and the volatility of the solvents is the prerequisite for this loss. The variation in vapor pressure of these compounds may thus influence skin absorption to a great extent. Information on the effect of vapor pressure, evaporation and ventilation on percutaneous absorption of organic solvents is apart from a few studies scanty in the literature. It is however clear that it is an important factor (Stewart and Dodd, 1964; Boman *et al*., 1995).

Toluene and *n*-butanol are representatives of two of the major solvent groups, aromatics and alcohols. They are extensively used individually or in mixtures and very often in situations where direct skin contact is possible, for example, as thinners for lacquers and paints, paint strippers and for cleaning brushes, and sometimes paint soiled skin. The lipophilic properties of organic solvents makes them effective in extracting epidermal lipids and thereby altering the barrier properties of the skin.

The present investigation documents the influence of ventilation the in vitro percutaneous absorption of 2 neat organic solvents, *n*-butanol and toluene and those solvents in binary mixtures with each other and in ternary mixtures with chloroform:-methanol (2:1).

MATERIALS AND METHODS

Chemicals

n-butanol, toluene, sodium chloride (analytical grade, Fisher Scientific, Pittsburgh, PA, U.S.A.), polyethylene glycol 20 oleyl ether (PEG20), Triton X-100 (Sigma Chemicals, St Louis, MO, U.S.A.), scintillation fluid (Universol™ or Ecolume™, ICN Radiochemicals, Irvine, CA, U.S.A.), ^{14}C-*n*-butanol and ^{14}C-toluene both labelled in the 1 position (Sigma Chemicals, 2.5 mCi/mmol and 2.63 mCi/mmol specific activity respectively). The labelled compounds were diluted with cold solvent to an activity of 1.25 mCi/ml.

Evaporation rate

Evaporation rate was measured using a gravimetric method described by Gilbert (Gilbert, 1971). Petri dishes with a diameter of 7.8 cm^2 was filled with 10 ml of solvent and placed on a scale. Each minute for 10 min the scale reading was recorded. The experiment was performed at room temperature (24°C) at a relative humidity of 45%.

Skin absorption

Human split-thickness (250 μm) thigh skin acquired from several sources at autopsy about 5 h after death (53 and 54 years old, Caucasian males, donors 1,2; 36 and 37 years old, Caucasian females, donors 3,4), stored frozen until use, was thawed one hour prior to use. The procedure of obtaining the skin was approved by the ethical committee for the School of Medicine at The University of California at San Francisco. The skin was mounted in all glass 1 cm^2, 3 ml flow through penetration/evaporation cells (LG-1084-SPC, Laboratory Glass Apparatus Inc., Berkeley, CA) (Spencer *et al*., 1979; Hawkins and Reifenrath, 1984; Reifenrath and Spencer, 1989; Gummer *et al*., 1987; Gummer and Maibach, 1987) (Fig. 1). The skin was allowed to acclimatize mounted in the cell 1 h prior to any exposures or measurements. Integrity of the skin membrane was checked with trans epidermal water loss (TEWL) (Evaporimeter EP1, Servomed, Kinna, Sweden) measured as described elsewhere (Abrams *et al*., 1991, 1993; Boman and Maibach, 1996; Nangia *et al*., 1998). Skin with a waterloss value less than 6 g/cm^2 × h was accepted.

The perfusion medium, normal saline solution, for the butanol exposures, or normal saline with 6% PEG20 added to increase solubility of the lipophilic solvent, for the toluene exposures, with a flow rate maintained at 2.5 ml/h (1.2 changes/h) (Cassette Pump, Manostat, New York, NY, U.S.A.), was stirred at 600 rpm mechanically in the cell to ensure sink conditions. The perfusion fluid was collected in 1 h intervals for at least 20 h, using a fraction collector (Retriever IV, Isco Inc., Lincoln, NJ, U.S.A.), into scintillation vials prefilled with 10 ml scintillation fluid to minimize evaporation of the studied solvent. Tracer marked solvent (200 or 300 μl) neat or in mixture, was applied to the skin surface and the ventilation tower applied. Ventilation was generated by coupling the air effluent end of the ventilation tower to the in house vacuum pump; the airflow was adjusted by individually regulated ball flowmeters. The ventilation flows studied for neat solvents were 0, 90, 400, and 900 ml/min. The mixtures were ventilated with 90 ml/min. The flowmeters were calibrated against an electronic flowmeter. The non-ventilated cells were covered with aluminum foil and sealed with Parafilm® to reduce evaporation and to ensure infinite exposure conditions.

The perfusion/absorption cells were maintained at 32°C with circulating water from a water bath.

Exposure was done with neat solvents, toluene or butanol, the two solvents mixed binary (50/50 v/v) and solvents mixed ternary with chloroform:methanol (2:1) (CM) in relation 50/50 (v/v) solvent/CM.

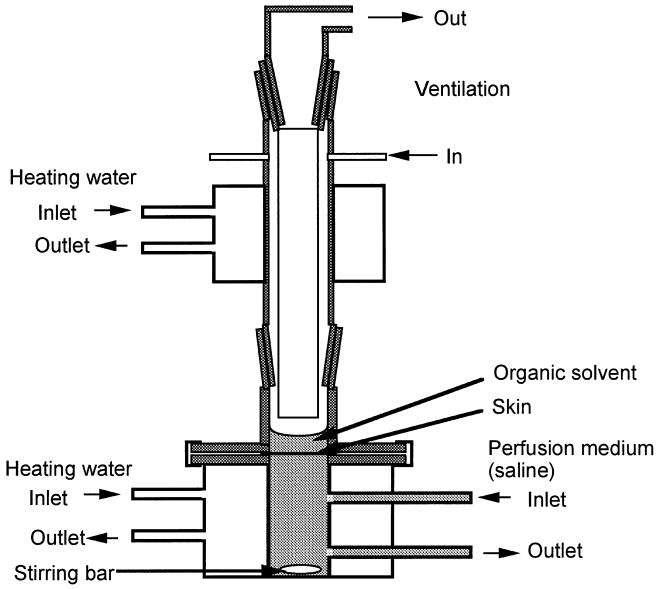

Fig. 1. Schematic drawing of ventilated in vitro skin absorption cell.

As control neat solvent, and mixtures under non ventilated conditions were studied. The evaporated solvents were not adsorbed to any adsorbant for technical reasons. Preliminary test found a break through when non-charcoal adsorbents were used and a severe quenching of scintillation when charcoal was tested.

At the end of the exposure the cells and skin were washed with surfactant solution and the skin digested in 4 ml Soluene 450 (Packard, Meriden, CT, U.S.A.). All samples were counted in a Packard Liquid Scintillation counter.

Statistical assessment of the data was done with *t*-test using Instat (GraphPad Software, San Diego) on a Macintosh computer. A p value $\leqslant 0.05$ was considered statistically significant.

RESULTS

The evaporation rate of neat toluene was $1.0 \pm 0.2 \times 10^{-5}$ g/cm^2 × s and for neat *n*-butanol $2.6 \pm 0.3 \times 10^{-6}$ g/cm^2 × s at room temperature under normal conditions.

The absorption profiles of neat *n*-butanol and toluene over human skin under non-ventilated and ventilated conditions in vitro are shown in Figs 2 and 3. Steady state absorption of toluene was estab-

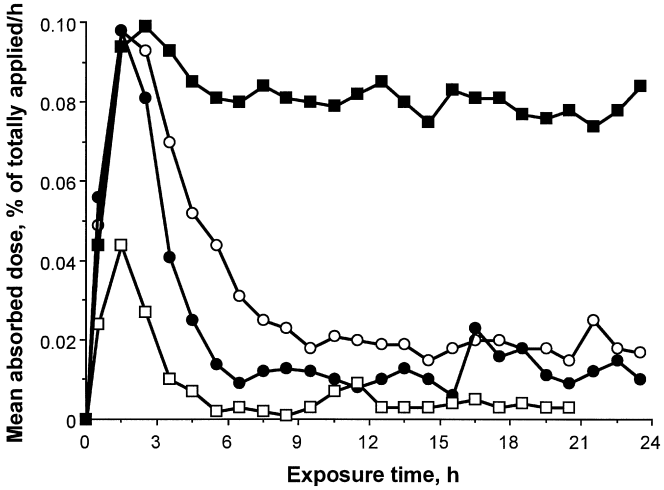

Fig. 2. Skin absorption profile of neat toluene under normal and ventilated conditions. Ventilation rate 0 (–■–) (n = 3), 90 (–○–) (n = 5), 400 (–●–) (n = 5), and 900 (–□–) (n = 5) ml/min.

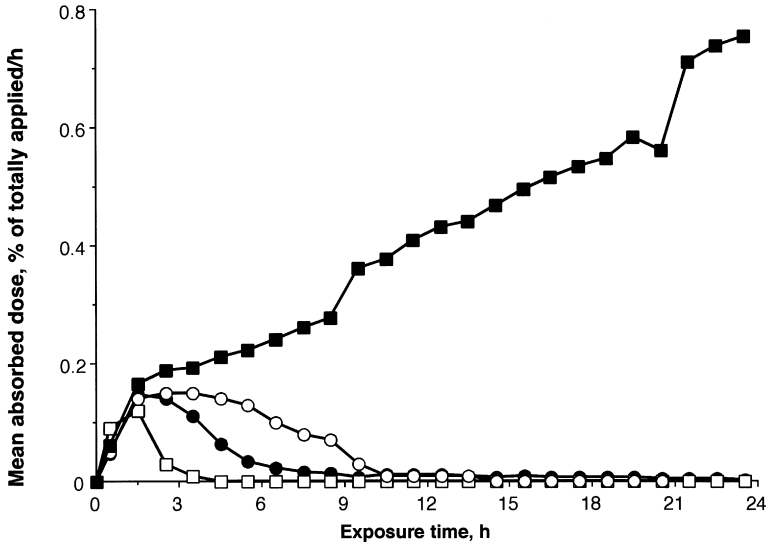

Fig. 3. Skin absorption profile of neat *n*-butanol under normal and ventilated conditions. Ventilation rate 0 (–■–) (n = 3), 90 (–○–) (n = 5), 400 (–●–) (n = 5), and 900 (–□–) (n = 5) ml/min.

lished at the non-ventilated exposure after 2 h exposure; for *n*-butanol steady state absorption was not attained during the time studied for the unventilated exposure. Exposure to both *n*-butanol and toluene were considered to be under infinite conditions in the unventilated situation since solvent remained in the depot at the end of exposure and the cumulative absorption profiles showed linear or exponential increase (Figs 4 and 5).

There was a clear interindividual variation in total absorption of the solvents between the skin donors; the absorption of toluene varied with a factor of 3.5 (1.1, 1.9 and 3.9% of total dose applied) and for *n*-butanol with a factor of 4.3 (2.2, 8.4 and 9.4% of total dose applied).

The ventilated exposures did not reach steady state for either solvent under any of the ventilation rates. The lowest ventilation rates generated the same initial absorption profiles as the non-ventilated exposure and then gradually decreased as the solvent evaporated. The cumulative absorption profiles demonstrate the influence of ventilation on percutaneous absorption with a decrease in slope of the lines (Figs 4 and 5).

The solvent absorbed during the exposure time was linearally related to the airflow rate (Fig. 6).

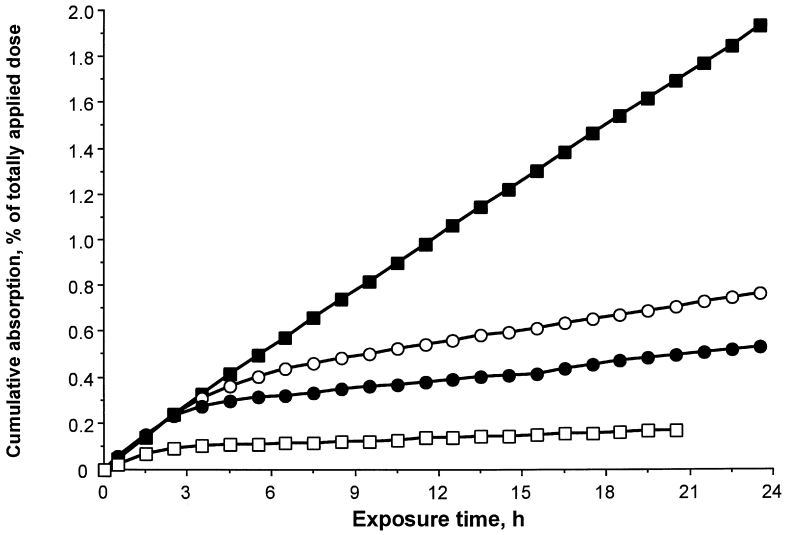

Fig. 4. Cumulative absorption of neat toluene under normal and ventilated conditions. Ventilation rate 0 (–■–) (n = 3), 90 (–○–) (n = 5), 400 (–●–) (n = 5), and 900 (–□–) (n = 5) ml/min.

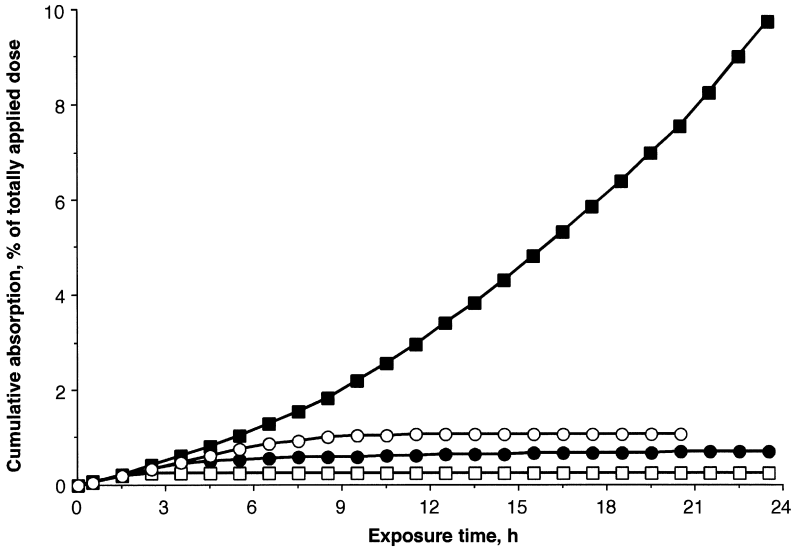

Fig. 5. Cumulative absorption of neat *n*-butanol under normal and ventilated conditions. Ventilation rate 0 (–■–) ($n = 3$), 90 (–○–) ($n = 5$), 400 (–●–) ($n = 5$), and 900 (–□–) ($n = 5$) ml/min.

The solvent with the highest volatility, toluene, gave a lower total absorption than *n*-butanol.

Exposure with the binary mixtures toluene/*n*-butanol resulted in a slight increase in absorption of toluene, 1.7 times (6.6 ± 0.2% of total dose applied), compared to the neat solvent (3.9 ± 0.5% of total dose applied) ($p < 0.001$) (Fig. 7, Table 1). The absorption of the *n*-butanol component in the mixture was considerably increased, 11.1 times (8.4 ± 2.8 vs 93.5 ± 5.2% of total dose applied) ($p < 0.001$) (Fig. 8, Table 1). The addition of C:M (2:1) to the solvents in ternary mixture resulted in a 2.8 times increase in total absorption of toluene (1.1 ± 0.3 vs 3.1 ± 0.5% of total dose applied) ($p < 0.001$) and 9.9 times increase in total absorption of *n*-butanol respectively (2.2 ± 1.1 vs 21.8 ± 9.3% of total dose applied) ($p < 0.001$) (Figs 9 and 10, Table 1). The influence of ventilation (90 ml/min) on all these exposures significantly reduced the total solvent absorbed from these mixtures (Figs 11 and 12, Table 1) compared to unventilated exposure.

DISCUSSION

Interest in the effect of evaporative loss of compounds during skin exposure has mainly concentrated on insect repellents (Spencer *et al.*, 1979; Reifenrath and Robinson, 1982). Their evaporation from the surface is functionally important for the

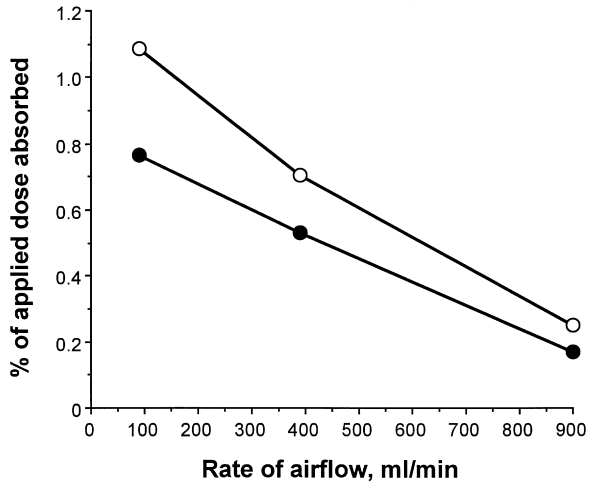

Fig. 6. Total absorbed dose through normal skin in vitro of *n*-butanol (–○–) and toluene (–●–) as a function of ventilation through normal skin in vitro.

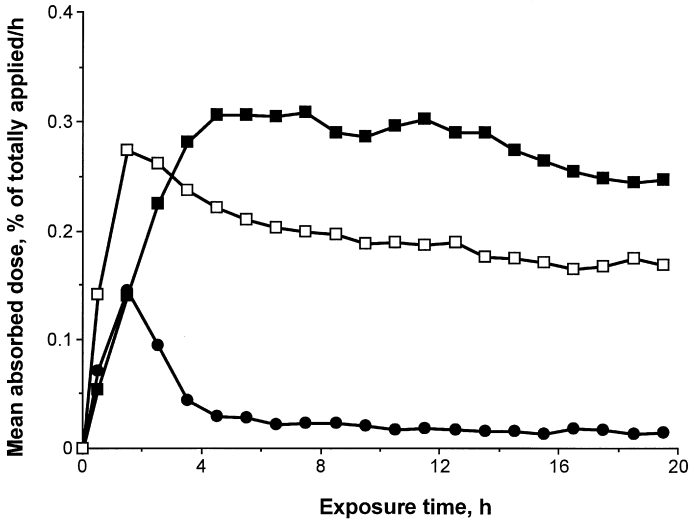

Fig. 7. Skin absorption profile of neat toluene and toluene in 50/50 mixture with *n*-butanol under normal and ventilated conditions. Neat solvent, unventilated exposure (–□–) (n = 6), toluene/butanol mixture, unventilated exposure (–■–) (n = 4), and toluene/butanol mixture, ventilated exposure 90 ml/min (–●–) (n = 5).

Table 1. Influence of ventilation air flow rate, skin donor and cosolvent on in vitro skin absorption of toluene and *n*-butanol

	Absorbed dose (% of total applied ± SD)									
	Toluene					*n*-Butanol				
	Donor 1	Donor 2		Donor 3		Donor 1	Donor 4		Donor 3	
Air flow (ml/min)	Neat	Neat	CM[a]	Neat	But[b]	Neat	Neat	CM	Neat	Tol[c]
0	1.9 ± 0.4	1.1 ± 0.3	3.1 ± 0.5	3.9 ± 0.5	6.6 ± 0.2	9.4 ± 4.9	2.2 ± 1.1	21.8 ± 9.3	8.4 ± 2.8	93.5 ± 5.2
90	0.8 ± 0.2	–	1.1 ± 0.3	–	0.7 ± 0.3	1.1 ± 0.6	–	2.0 ± 0.5	–	10.5 ± 11.4
400	0.5 ± 0.1	–	–	–	–	0.7 ± 0.3	–	–	–	–
900	0.2 ± 0.03	–	–	–	–	0.3 ± 0.1	–	–	–	–

[a]CM = 50/50 mixture with chlorform/methanol (2:1).
[b]But = 50/50 mixture with butanol.
[c]Tol = 50/50 mixture with toluene.

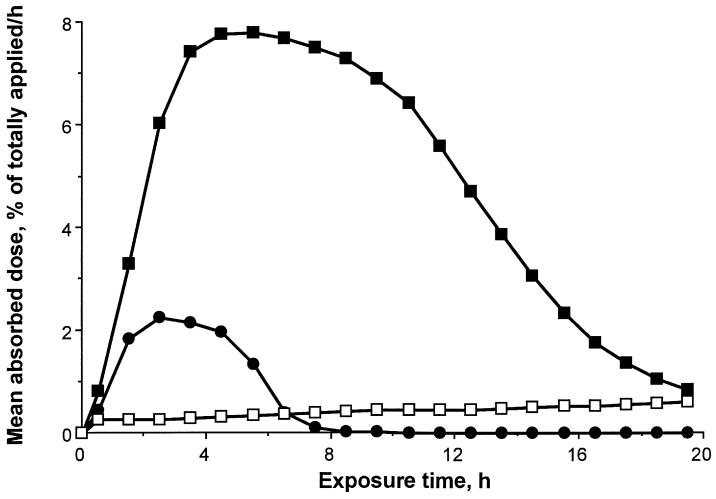

Fig. 8. Skin absorption profile of neat *n*-butanol and *n*-butanol in 50/50 mixture with toluene under normal and ventilated conditions. Neat solvent, unventilated exposure (–□–) (n = 5), butanol/toluene mixture, unventilated exposure (–■–) (n = 5), and butanol/toluene mixture, ventilated exposure 90 ml/min (–●–) (n = 6).

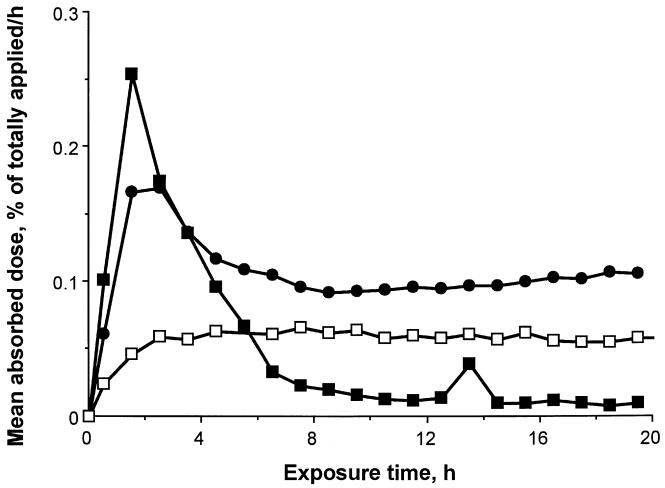

Fig. 9. Skin absorption profile of neat toluene and toluene in 50/50 mixture with chloroform:methanol (2:1) under normal and ventilated conditions. Neat solvent, unventilated exposure (–□–) (n = 5), toluene/chloroform:methanol mixture, unventilated exposure (–●–) (n = 5), and toluene/chloroform:methanol mixture, ventilated exposure 90 ml/min (–■–) (n = 5).

insect repelling-process and a minimum effective dose of insect repellent can be established which is a balance of effect and duration of evaporation from the intentionally applied compound on the skin (Gabel *et al.*, 1976). However, in the occupational environment the intention is to minimize chemical exposure that may be harmful to the skin and systemically when absorbed. Evaporative loss of compound from the skin can be a significant route of loss if it has a sufficiently high vapour pressure and the evaporation is not impeded by full or semi-occlusion. Little emphasis has been placed on this aspect in occupational hygiene and very few, occupationally important compounds have been studied in detail (Hawkins and Reifenrath, 1984; Reifenrath and Spencer, 1989). Occupational skin exposure to chemicals is often brief and at irregular intervals during a work shift. This exposure pattern does not usually favour the use of protective gloves. Unless gloves are donned from the start of a work shift, they often seems too much bother to use during critical exposure times, and thus the skin is exposed briefly to defatting and irritant chemicals at various intervals. Industrial exposure is also often to mixtures and seldom to the neat compound or solvent. If one or several compounds are

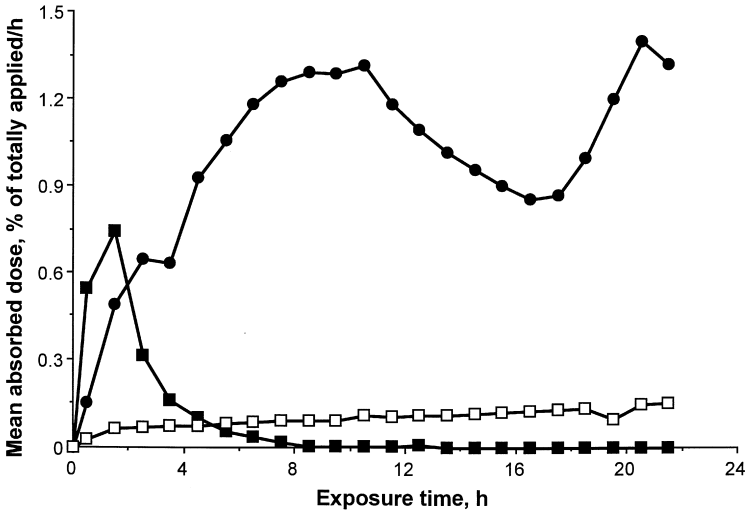

Fig. 10. Skin absorption profile of neat n-butanol and n-butanol in 50/50 mixture with chloroform:methanol (2:1) under normal and ventilated conditions. Neat solvent, unventilated exposure (–□–) (n = 6), butanol/chloroform:methanol mixture, unventilated exposure (–●–) (n = 6), and butanol/chloroform:methanol mixture, ventilated exposure 90 ml/min (–■–) (n = 5).

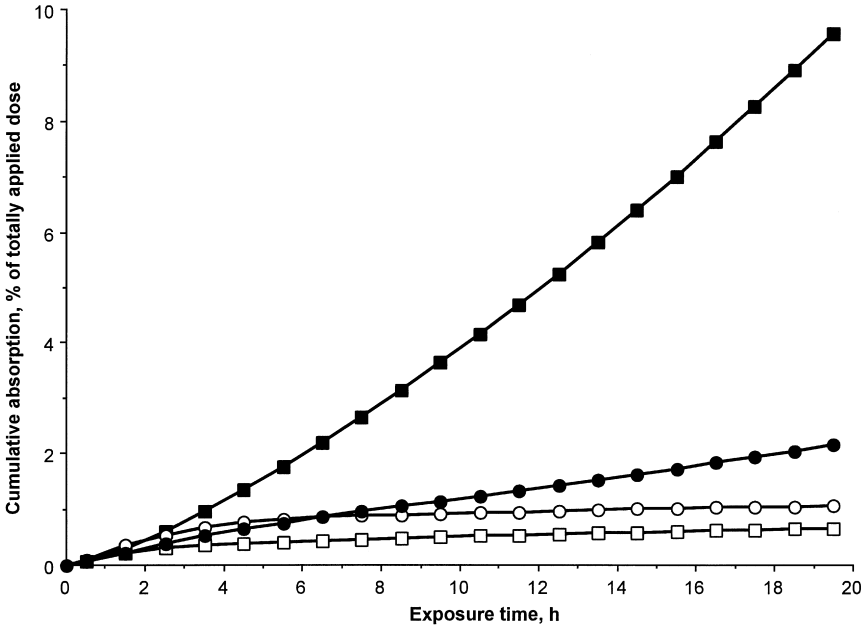

Fig. 11. Cumulative absorption of toluene from 50/50 mixture with *n*-butanol and from 50/50 mixture with chloroform:-methanol (2:1) under normal and ventilated conditions. Toluene/butanol mixture, unventilated exposure (–■–) (n = 4), and toluene/butanol mixture, ventilated exposure 90 ml/min (–□–) (n = 5). Toluene/chloroform:methanol mixture, unventilated exposure (–●–) (n = 5), and toluene/chloroform:methanol mixture, ventilated exposure 90 ml/min (–○–) (n = 5).

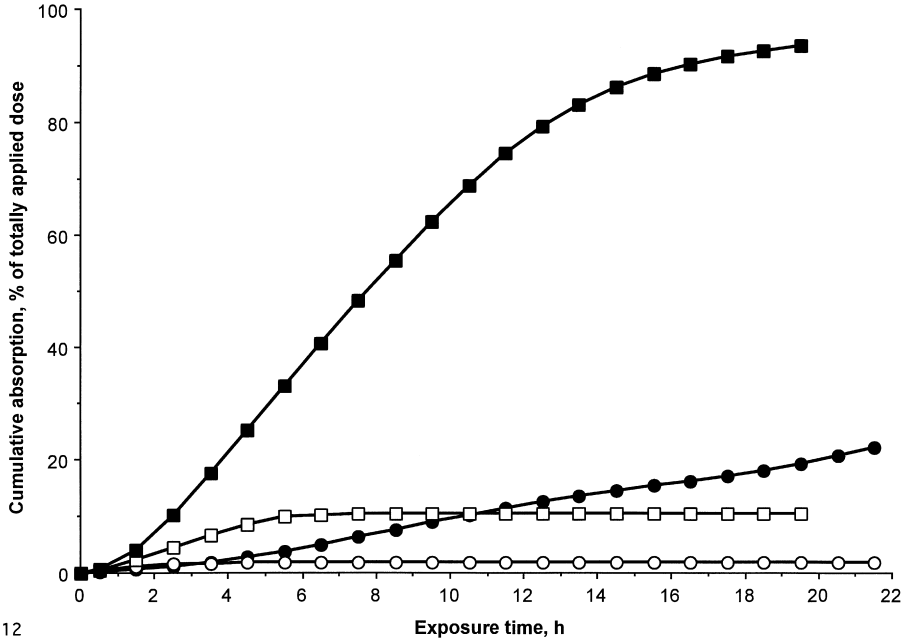

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Fig. 12. Cumulative absorption of *n*-butanol from 50/50 mixture with toluene and from 50/50 mixture with chloroform:-methanol (2:1) under normal and ventilated conditions. Butanol/toluene mixture, unventilated exposure (–■–) (n = 5), and butanol/toluene mixture, ventilated exposure 90 ml/min (–□–) (n = 6). Butanol/chloroform:methanol mixture, unventilated exposure (–●–) (n = 6), and butanol/chloroform:methanol mixture, ventilated exposure 90 ml/min (–○–) (n = 5).

volatile, evaporative loss of one or several of these can dramatically change the absorption of the others as their relative concentration are increased.

Many organic solvents have a high vapour pressure and can be expected to have a substantial loss through evaporation when non-occluded skin is exposed (Boman, 1996). The influence on skin absorption of evaporative loss of chemicals with various vapour pressures has been discussed (Hawkins and Reifenrath, 1984, 1986; Reifenrath and Spencer, 1989). Reifenrath studied the evaporative loss and in vivo percutaneous absorption and found that it ranged from a few percent for compounds with low vapour pressure to over 50% of the dose applied for those with the highest vapour pressures. Although the relationship was not linear the results agrees with ours.

Both solvents in our study have moderately high vapour pressures (28.4 mmHg toluene, 4.39 mmHg *n*-butanol) and are lost by evaporation from the skin at relatively high rates. The evaporation rates from an open surface into ambient air was $(1.0 \pm 0.2) \times 10^{-5}$ g/cm^2 × s for toluene and $(2.6 \pm 0.3) \times 10^{-6}$ g/cm^2 × s for butanol and is in accord with literature data: 1.9×10^{-5} g/cm^2 × s and 3.4×10^{-6} g/cm^2 × s respectively (Larson, 1968). The evaporation study was performed in an ordinary laboratory and the discrepancies from the literature data may be due to variations in ventilation, room climate and evaporation vessel. It can be assumed that the loss from the skin surface is higher when the skin temperature is higher than the surrounding air at which temperature these experiments were performed. No attempt was made to measure the evaporated amounts or evaporation rates during the in vitro skin exposure. The exposure with relative large amounts of solvent with high evaporation rates made sampling with adsorbent difficult as the adsorbent was saturated in a short time. Preliminary tests with charcoal gave unacceptable quenching in the scintillation analysis.

The evaporative loss of a volatile compound from a non-occluded skin surface can be anticipated and an influence of ventilation rate may be operative. The results show that this was the case; there was a clear relationship between the ventilation rate and the amount of solvent absorbed (Fig. 6) with a significant reduced absorption for both solvents at all ventilation rates compared to non-ventilated exposure. The highest ventilation rate gave fast evaporation, which was seen as a rapid decrease in absorption. Steady state of absorption was not reached during the ventilated exposures for any of the solvents or mixtures studied. The importance of ventilation rate on evaporation from the skin surface has also been studied by Reifenrath (Reifenrath and Spencer, 1989) using diethyltoluamide as model compound. In the ventilation rate they used — 30 and 60 ml/min — evaporation increased from 45 to 54% of total applied dose with a concurrent reduction of amount absorbed from 20% to 15%. Smith's data also corroborates ours and has implications for occupational hygiene: evaporation curves of diethyltoluamide were analogous to the absorption curve for our solvents (Smith *et al*., 1963). A constant evaporation rate was observed for 15 h followed by a sudden and marked drop when the majority of compound was evaporated/absorbed. This closely resembles our absorption profiles, albeit, the time frame for the solvents are much shorter as they are more volatile.

The nature of the flow across the solvent surface in the absorption studies was not assessed but can not be considered laminar as the air inlet is perpendicular to the skin surface following the design of the test cell. Airflow was measured as volume changes/time (ml/min) rather than air velocity (m/s).

Mixing solvents with disparate lipophilic and hydrophilic properties will result in a mixture with different solving properties and accordingly a different lipid dissolving ability. This is often used in industry to be able to dissolve greasy and non-greasy dirt and to be able to formulate solutions of compounds with varied lipophilic properties. The 2:1 mixture of chloroform and methanol is often used as a means to extract the lipids from various biological tissues including skin (Folch *et al*., 1957, Swartzendruber *et al*., 1987). The exposure to butanol in binary mixture with toluene and ternary mixture with chloroform:methanol (2:1) significantly increased the solvent absorption. Ventilating the mixture exposure with 90 ml/min airflow significantly reduced the absorption. However, the total amount absorbed was of the same magnitude as exposure to neat solvent under non-ventilated conditions for 20 h. The same tendency was observed for the other compound in the mixture, toluene, although the increase in absorption was not as marked as for butanol — a result that was found in in vivo studies on guinea pigs (Boman, 1989).

One factor that influences percutaneous absorption is the interindividual variation in the results due to the normal variation in the human skin barrier properties. Skin from all 4 donors was not subjected to all types of treatment due to shortage of material. However, of the tested combinations there was a significant interindividual variability in absorption of the neat solvents in our experiments. It varied up to as much as 4 times and a statistically significant difference between two of the three donors at the butanol exposure and between all three at the toluene exposure was seen. This interindividual variation in absorption rate of the studied solvents is somewhat larger than what has been recorded for water. Permeability coefficient for 33 donors averaged $1.55 \pm 0.08 \times 10^{-3}$ cm/h with an interindividual variability factor of 3.2 (Bronaugh *et*

al., 1986). The skin material was acquired as post mortem grafts and it was not possible to obtain any dermal history of the donors and minor variations in the barrier properties not detectable with the pre-exposure barrier test may account for this variation.

Ventilation in industrial localities is according to Swedish occupational health regulations measured in volume changes per time and has to be at least 7 l./s, with additional increase if emissions are present. However, in laboratory fume hoods it is measured as a linear flow and has to be at least 0.5 m/s in the opening slit during normal operating conditions (Anonymous, 1993, 1997). This supply of clean air will not only provide clean breathing air but will also reduce skin absorption as the evaporation of solvent from an unoccluded skin surface is increased at ventilation. The evaporation is also important in reducing the skin absorption as most exposures to the skin in industry are intermittent and volatile compounds evaporate freely between exposures (Boman *et al*., 1995).

CONCLUSION

Based on the presented results it is evident that skin absorption of volatile organic solvents at continuous ventilated conditions is related to their volatility and to the ventilation rate. The presented data and earlier studies suggest that maintaining an adequate workplace ventilation is an important occupational hygienic measure not only to reduce exposure via respiration but also to reduce absorption via the skin of volatile compounds particularly as the skin exposure generally is intermittent and the solvents evaporates between exposures.

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