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(Article begins on next page)

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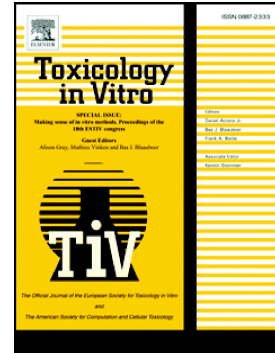
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Skin contamination as pathway for nicotine intoxication in vapers

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Abstract

Growing warnings on health effects related to electronic cigarettes have met inconclusive findings at present. This study analyzed the in vitro percutaneous absorption of nicotine resulting by skin contamination with two e-liquids (refill 1 and 2) containing nicotine at 1.8%. Donor chambers of 6 Franz cells for each refill liquid were filled with 1 mL of nicotine e-liquid for 24 hours; at selected intervals, 1.5 mL of the receptor solutions were collected for nicotine concentration analysis by mean gas chromatography-mass spectrometry (LOD: 0.01 µg/ml). The experiment was repeated removing the nicotine donor solution after 10 minutes from the application and rinsing the skin surface three times with 3.0 ml of milliQ water. A total of 12 cells with 24 hours exposure and 12 cells washed were studied. The mean concentration of nicotine in the receiving phase at the end of the experiment was 54.9±29.5 and 30.2 ± 18.4 µg/cm^2 for refill 1 and 2 respectively and significantly lower in washed cells (4.7±2.4 and 3.5±1.3 µg/cm^2). The skin absorption of nicotine can lead to minor health ilness in vapers, while caution must be paid to dermal contamination by e liquids in children. The skin cleaning significantly reduced the transdermal absorption kinetic and intradermal deposition of nicotine.

Introduction

The explosive growth of the global electronic cigarette (e-cig) market has been accompanied by increasing attention to the potential health hazards of this new technology. Chemical, toxicological, and clinical studies are inconclusive at present, so the debate between proponents and opponents of e-cigs is still open (Orr MS., 2014). One of the most persuasive matters for the opposition is that the e-cig, originally marketed as a tobacco reduction or smoking cessation product, does not reduces nicotine addiction in conventional smokers (Popova et al., 2013; Grana et al., 2014); furthermore, use of e-cigarettes may encourage conventional cigarette use among adolescents (Dutra et al., 2014).

E-cigs are devices designed to deliver nicotine via the lungs by vaporization of refill liquids (e-liquid) where nominal mean nicotine concentrations range from 0 to 3.6 % (Cameron et al., 2014); however Davis et al. (2014) report that 65% of products had nicotine concentration deviating for more than 10% from the manufacturers' labels.

Nicotine is a water and lipid soluble alkaloid readily absorbed through all routes of exposure. Poisonings caused by accidental ingestion of e-liquids containing a nicotine concentration of 1.8% (Basset et al., 2014) or 2.4% (Gill et al., 2015) have been reported in children. Nicotine is a weak base, easily absorbed through the skin (Schevelbein et al., 1972): transdermal penetration has been used as a smoking reduction and cessation aid in the last thirty years (Rose et al., 1984), while skin contamination has been reported as a pathway for intoxication in tobacco harvesting (Curwin et al., 2005). After dermal patch application, nicotine is gradually absorbed through the skin, plasma levels rise slowly over 6 to 10 hours, remain at steady level for about 7 to 8 hours, and decline during the final 6 hours (Zevin et al., 1998). Moreover, mild systemic symptoms of nicotine poisoning were documented after skin contact with nicotine refill solution or leaking nicotine from cartridges (Cantrell and Clark, 2014). Other cases were reported in the past for unintentionally spilled nicotine solutions onto the skin in children (Won Kim and Baum, 1975). Recently, Maina et al. (2016) studied in in-vitro experiments nicotine absorption through the skin contaminated with

refill e-liquid, finding very low values (3.04 mg at 24 h after skin contamination of 100 cm^2) compared to nicotine delivered by transdermal patches (Benowitz, 1995).

This study aims to assess the in vitro transdermal absorption of nicotine contained in two e-liquids marketed by different producers and the effect of a skin cleaning procedure.

2. Materials and methods

2.1 Chemicals

All the chemicals used of analytical grade were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Water reagent grade was produced with a Millipore purification pack system (milliQ water). The physiological solution used as receptor fluid was freshly prepared by dissolving 9.00 g of NaCl, 2.38 g of Na_2HPO_4 and 0.19 g of KH_2PO_4 into 1.00 L of milliQ water (final pH 7.35). The concentration of the salts in the receptor fluid was approximately the same as that present in blood. The two refill liquids for e-cigarettes, used as donor phase, are available in the market. The nominal nicotine concentration of 18 mg ml^{-1} was confirmed by gaschromatography – mass spectrometry (GC-MS). Both refill had pH of 7.9 and pKa of nicotine is 8.0 (Gorrod et al. 1999).

2.2 In vitro experiments

The experiments were performed using human cryopreserved skin (Castagnoli et al., 2003) from the back of three donors (two males and one female). Before use, the skin was left to thaw gradually to room temperature. The epidermal side of the skin was exposed to room conditions, while the dermal side was bathed in physiological solution. From each skin specimen, 4 cm x 4 cm pieces were cut and mounted separately on the diffusion cells. Each piece of skin was clamped between the donor and the receptor compartment; the exposed skin area was 3.29 cm^2 and the average skin thickness was 0.7 mm. Skin integrity was assessed using the Trans Epidermal Water Loss (TEWL) method as described by Guth et al. (2015). Cells with a value >10 g m^{-2} h^{-1} were considered to be damaged and rejected.

The experiments were carried out as follows:

Experiment I:

Donor chambers of 6 static Franz (1975) cells (two cells for each skin donor) for each refill liquid were filled with 1 mL of nicotine e-liquid providing an amount of 5.5 mg of nicotine per cm^2 of skin, in order to ensure an infinite dose. The receptor compartment, filled with physiological solution, had a mean volume of 14.0 mL and was maintained at +32°C by the circulation of thermostated water in the jacket that surrounds the cell. The solution in each cell was continuously stirred using a Teflon-coated magnetic stirrer. At selected intervals, 0.5, 1, 2, 4, 6, 8, 16, and 24 hours, 1.5 mL of the receptor solutions were collected for nicotine concentration analysis. Each receptor sample was immediately replaced with an equal volume of fresh physiological solution. At 24 h, the donor phases and the receiving solutions were removed and stored in the freezer. The skin samples were removed from the Franz cells, rinsed three times with 3 mL of milliQ water and then exposed surfaces were cut with surgical scissors and stored in the freezer for further analysis. The rinsing milliQ water aliquots were added to the donor phases before freezing.

Experiment II

A second experiment was carried out in order to mimic the washing after a contamination. The exp. 1 was repeated but after 10 minutes from the application, the nicotine donor solutions were removed and the skin surfaces were rinsed three times with 3.0 mL of milliQ water. Then, 1 mL of physiological solution was added to each donor chamber, in order to maintain the skin in the same hydration conditions of the experiment I and of the blank cells (Larese et al., 2008; Nesvadbova et al., 2015). The washing solutions were collected for following analysis.

Blanks

Four cells were added as blank using physiological solution as donor solution instead of nicotine e-liquid.

Summarizing, the replicates were organized taking into account 3 skin donors, 2 refill liquids and 2 different treatments, for a total of 24 exposed cells and 4 blanks.

As the equipment used in the tests was static, there is no relationship among the cells; hence each of them represents an independent evaluation.

2.3 Analytical measurements

Aqueous solution of nicotine was extracted with SPE C18 and eluted with methanol (1 mL). The recovery of the extraction, calculated from 10 standards of certified reference material (nicotine 99%, Acros Organics) was 96,4%. The standard deviation of the measured extract was 0.3.

Extract was analyzed with gaschromatography-mass spectrometry (Thermofisher mod. Trace-Polaris Q) in single ion monitoring mode (ION 133 and 161). The column used was 5% phenyl-95% Metilsilicone, 30 m length, 0.25 mm internal diameter, 0.25 µm film thickness. The injection, in PTV mode, for all samples was 2 µl. The sample was analyzed from 50°C to 150°C with a rate of 5°C min^{-1} and then from 150°C to 270°C with a rate of 20°C min^{-1}.

The limit of quantification for nicotine was 0.05 µg mL^{-1} with a limit of detection of 0.01 µg mL^{-1}. Nicotine in skin was extracted with methanol (1 mL) for 16 hours. Extract was analyzed with gaschromatography-mass spectrometry with the same conditions described above.

2.4 Data analysis

Nicotine concentration data (mg cm^{-3}) in the receptor solution were converted to the total amount that permeated through the skin (mg cm^{-2}), with a correction for dilution due to sampling removal. Flux (mass/area–time or µg cm^{-2} h^{-1}) was determined for each cell from the slope of the linear part of the plot of cumulative chemical mass per unit area in the receptor solution over time. Lag time (h) was estimated as the point where the flux curve intercepts the x-axis.

Data were reported as mean and standard deviation. Statistical differences between results in cells washed and not washed, donors and refills were tested by means of ANOVA. A p value of 0.05 was considered as significant.

3. Results

Table 1 summarizes the nicotine permeation data.

Nicotine was able to permeate the skin with a flux of 2.92 ± 1.53 µg cm^{-2} h^{-1} and of 1.58 ± 0.96 µg cm^{-2} h^{-1} after the exposure to refill 1 and 2 solutions, respectively, with no statistical differences between them (replicate n. 12; F:0.5, p>0.05). Lag times were similar (F: 1.9, p>0.05) and nicotine

inside the skin at the end of experiments was comparable (F: 4.22, p>0.05). No differences were shown between donors.

Nicotine skin absorption reduced significantly (p<0.001) after the washing procedure with a flux of 0.29 ± 0.20 µg cm^{-2} h^{-1} and of 0.19 ± 0.07 µg cm^{-2} h^{-1} in cells treated with refill 1 and 2 solutions, respectively, while no differences were found for lag times between washed and non-washed cells.

Figure 1 provides permeation profile of nicotine. Nicotine was not detectable in the receiving phase up to 2 hours after the start of the experiment; it slowly increased between 4 and 6 hours, then gradually increased reaching the concentration of 54.9 ± 29.5 and 30.2 ± 18.4 µg cm^{-2} for refill 1 and 2 solutions treated cells, respectively.

The permeation profile for the cleaned skin followed the same trend reaching, as expected, lower values at the end of the experiment (4.7 ± 2.4 and 3.5 ± 1.3 µg cm^{-2} for refill 1 and 2, respectively).

4. Discussion

The main finding of this study is that nicotine can be absorbed through the skin contaminated by refill liquids, and that absorption is detectable also when the skin is washed for 10 minute after the contact with the liquid.

From the toxicological point of view, our results are important to evaluate if skin contamination with e-cig refills could be dangerous for human health. The contamination of the body surface area due to e-liquid spilled onto the skin of the vaper can be estimated between 170 cm^2 (area of a palm) and 340 cm^2 (area of the hand), depending on the volume of the e-liquid package (10 and 20 mL, respectively). Taking into account that the intravenous administration of 5 mg of nicotine leads in adults to only minor adverse effects (coughing, and nausea) (Henningfield et al.,1983), the contamination of the skin area should account for 100 cm^2 (considering our maximum results for 24 hours penetration of 54.9 microg/cm^2) to achieve the above toxicological level of concern. This result suggests that the contamination of even small skin area by e-liquid at 1.8% of nicotine can be associated with minor health illness. However, the area of the skin contaminated needed to cause the severe health effects related to the toxicological threshold value in adults (500 mg), proposed by Mayer (2010), is about 10000 cm^2 (corresponding to half of the body surface): it is clear that this scenario is completely unrealistic. Likewise, in the case of a child of 10 Kg, where the mean estimated transdermal nicotine dose for symptomatic children is 1.8 g. (Wolf et al., 1997), the contaminated skin surface is about 30 cm^2 (half palm). Moreover, even in the case of children, the contamination of the skin surface required to reach the lethal dose (70 mg) is about 20% of the BSA, corresponding to the area of the chest.

Our study for the first time analyzed also the effect of the skin washed after contamination, adding important information on nicotine skin absorption, when a washing procedure is used after skin contamination. Despite the total amount of nicotine that passed through the skin is significalntly reduced compared to not decontaminated skin (p<0.001), the permeation profile is comparable to the not rinsed skin (the nicotine is detected after 2 hours following the start of the

experiment and continue to rise until reaching 4.05 ± 1.08 µg cm^{-2} at the end of the experiment). This means that even a short contact time (10 min) produces trandermal absorption of nicotine suggesting the need to clean the skin as soon as possible when the contamination occurs. The effectiveness of the cleaning procedure was demonstrated by the absence of nicotine into the skin washed while nicotine was present in not cleaned skin (2.20 ± 1.23µg cm^{-2}). This finding confirms that the skin can act as a reservoir able to deliver nicotine.

Results are in accord with our previous study (Maina et al., 2016) when we tested a different refill with lower nicotine content (0.8% vs 1.8% used in the present study), nevertheless using a higher nicotine concentration we obtained a lower flux through the skin. Indeed, Zorin et al. (1999) documented that the nicotine permeation increases in a linear relationship with its concentration in acqueos solutions in the range 1-10%, then we would have expected a higher nicotine skin absorption using a nicotine concentration of 1.8% than that obtained using a nicotine concentrations of 0.8%. A plausible explanation for the observed differences in nicotine fluxes magnitude could be related to the different characteristics of the skin used in the two experiments. It is well known that the percutaneous penetration of chemicals is affected by the biological characteristics of the skin (anatomical site, age and sex of the skin's donor) as well as by the chemical properties of the donor solution made by nicotine and diluent used. A change in pH of the solution in contact with the skin (Nair et al., 1997) and a variation in nicotine ionization state can modify skin absorption (Kuswahyuning and Roberts, 2014). In addition, nicotine permeation profile reported in literature referred to aqueous solutions of nicotine, while the refill liquids contain chemicals (i.e. propylene glycol, flavours) that could affect absorption kinetics of the nicotine through the skin (Aungst, 1988).

Our study has some limitations that need to be considered. First of all, we did not considered nicotine metabolism that can happen into the skin, thus our results can underestimate real scenario; secondly, the high hydration of the skin applying 1 mL of physiological solution after the washing procedure can enhance nicotine skin absorption (Ishida et al., 2015), thus overestimate real

scenario. Moreover, it should take into account that the toxicological evaluation relies on experimental results claiming/ describing a worstcase scenario. Nevertheless, our study investigated the important topic of nicotine transdermal penetration following skin contamination in vaper, increasing knowledge on the widespread use of liquid nicotine and the easy contamination of the skin that can happen during normal procedures.

5. Conclusions

The skin absorption of nicotine can lead to minor health ilness in vapers, while caution must be pay to dermal contamination by e liquids in children The early cleaning of the skin further reduces the nicotine transdermally absorbed.

Conflict of interest statement
None.

References

Aungst, B. 1988. Nicotine skin penetration characteristics using aqueous & non-aqueous vehicles, anionic polymers, and silicone matrices. Drug. Dev. Ind. Pharm. 14(11),1481.

Basset, R.A., Osterhoudt, K., Brabazon, T., 2014. Nicotine poisoning in a infant. N. Engl. J. Med. [eLetter] 5 June 2014. doi: 10.1056/NEJMc1403843.

Benovitz, N.L., 1995. Nicotine patches. BMJ. 310, 1409–1410.

Cameron, J.M., Howell, D.N., Whitem J.R., Andrenyak, D.M., Layton, M.E., Roll, J.M., 2014. Variable and potentially fatal amount of nicotine in e-cigarettes nicotine solutions. Tob. Control. 23,77-78.

Cantrell, F.L., Clark, R.F., 2014. More on nicotine poisoning in infants. N.Engl.J.Med.371,880.

Castagnoli, C., Alotto, D., Cambieri, I., Casimiri, R., Bluffi, M., Stella, M., Teich Alasia, S., Magliacani, G., 2003. Evaluation of donor skin viability: fresh and cryopreserved skin using tetrazolium salt assay. Burns. 29,759–767. doi:10.1016/j.burns.2003.01.001.

Curwin, B.D., Hein, M.J., Sanderson, W.T., Nishioka, M.G., Buhler, W., 2005. Nicotine exposure and decontamination on tobacco harvesters' hands. Ann. Occup. Hyg. 49,407-413.

Davis, B., Dang, M., Kim, J., Talbot, P., 2014. Nicotine concentrations in electronic cigarette refill and do-it-yourself fluids. Nicotine Tob. Res. doi: 10.1093/ntr/ntu080.

Dutra, L.M., Glantz, S.A., 2014. Electronic cigarettes and conventional cigarette use among US adolescents: a cross-sectional study. JAMA pediatrics. 168, 610-617.

Franz, T.J., 1975. Percutaneous absorption on the relevance of in vitro data. J. Invest. Dermatol. 64(3),190–195. doi:10.1111/1523-1747.

Gill, N., Sangha, G., Poonal, N., Lim, R., 2015. E-cigarette liquid nicotine ingestion in a child: case report and discussion. CJEM. 1-5. doi: 10.1017/cem.2015.10.

Gorrod, JW.; Jacob, P, III. Analytic Determination of Nicotine and Related Compounds and Their Metabolites. Elsevier; New York: 1999.

Grana, R., Benowitz, N., Glantz, S.A., 2014. E-cigarettes: a scientific review. Circulation 129, 1972-1986.

Guth K, Schäfer-Korting M, Fabian E, Landsiedel R, van Ravenzwaay B., 2015. Suitability of skin integrity tests for dermal absorption studies in vitro. Toxicol in Vitro.29,113-123. doi: 10.1016/j.tiv.2014.09.007.
Henningfield, J.E., Miyasato, K., Jasinski, D.R, 1983. Cigarette smokers self-administer intravenous nicotine. Pharmacol. Biochem. Behav. 19: 887–890.

Ishida, M., Taheuchi, H., Endo, H., Yamaguchi, J.I., 2015. Impact of humidity on in vitro human skin permeation experiments for predicting in vivo permeability. J. Pharm. Sci. 104, 4223-4231.

Kuswahyuning, R., Roberts, R., 2014. Concentration dependency in nicotine skin penetration flux from aqueous solutions reflects vehicle induced changes in nicotine stratum corneoum retention. Pharm. Res. 31,1256-1264.

Larese Filon, F., D'Agostin, F., Crosera, M., Adami, G., Bovenzi, M., Maina, G. 2008. In vitro percutaneous absorption of chromium powder and the effect of skin cleanser. Toxicol. in Vitro. 22, 1562–1567.

Maina, G., Castagnoli, C., Passini, V., Crosera, M., Adami, G., Mauro, M., Larese Filon. F. 2016. Transdermal nicotine absorption handling e-cigarette refill liquids. Regul. Toxicol. Pharmacol. 74,31–33.

Mayer, B., 2014. How much nicotine kills a human ? Tracing back the generally accepted lethal dose to dubious self-experiments in the nineteenth century. Arch. Toxicol.88,5-7.

Nair, M.K., Chetty, D.J., Ho, H., Chien, Y.W., 1997. Biomembrane permeation of nicotine: Mechanistic studies with porcine mucosae and skin. J. Pharm. Sci. 86(2),257–62.

Nesvadbova, M., Crosera, M., Maina, G., Larese Filon, F. 2015. Povidone iodine skin absorption: An ex-vivo study. Toxicol. Lett. 235,155–160.

Orr, M.S., 2014. Electronic cigarettes in the USA: a summary of available toxicology data and suggestions for the future. Tob. Control.23:ii18-ii22 doi:10.1136/tobaccocontrol-2013-051474.

Popova, L., Ling, P.M., 2013. Alternative tobacco product use and smocking cessation. A national study. Am.J.Pub.Health.103, 923-930.

Rose, J., Jarvik, M.E., Rose, K., 1984. Transdermal administration of nicotine. Drug. Alcohol.Depend. 13(3),209–13.

Schevelbein, H., 1972. Nicotine. Encyclc. Occup. Health Safety. 2, 933 – 934.

Zevin, S., Gourlay, S.T., Benowitz N.L., 1998. Clinical pharmacology of nicotine. Clin. Pharmacol. 16,557-564.

Zorin, S., Kuylenstierna, F., Thulin, H., 1999. In vitro test of nicotine's permeability through human skin. Risk evaluation and safety aspects. Ann. Occup. Hyg. 43, 405e413. http://dx.doi.org/10.1016/S0003-4878(99)00030-7.

Wolf, A., Burkhart, K., Caraccio, T., Litovitz, T.,1997. Childhood poisoning involving transdermal nicotine patches. Pediatrics. 99: 5. http://www.pediatrics.org/cgi/content/full/99/5/e4

Won Kim, J.i., Baum, C.R., 2015. Liquid nicotine toxicity .Ped. Emer. Care. 31, 517-531.

Figure 1: permeation profiles : means and standard deviations of nicotine amount ($\mu g\ cm^{-2}$) in human cryopreserved skin
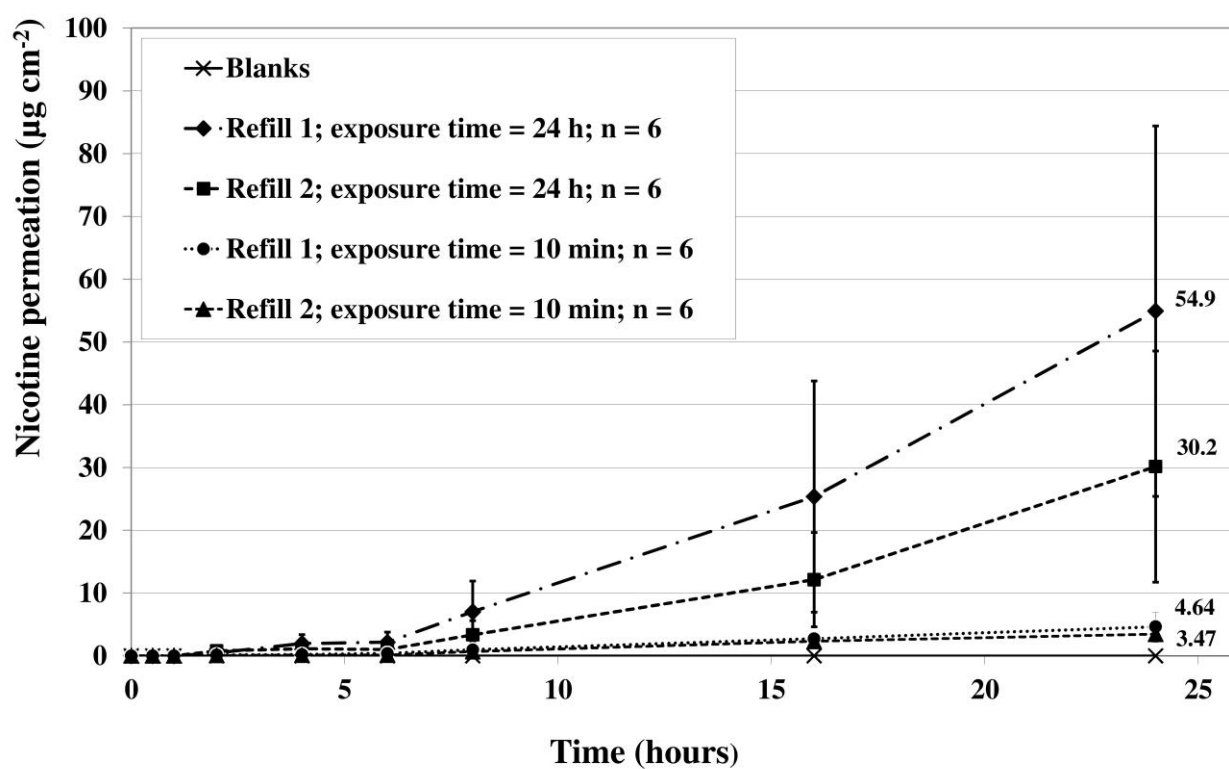
Table 1: Nicotine skin permeation data (mean ± ds)

	Refill 1	Refill 2
Flux (μg cm^{-2} h^{-1})	2.92 ± 1.53	1.58 ± 0.96
	0.29 ± 0.20[§]	0.19 ± 0.07[§]
Lag time (h)	6.3 ± 1.3	6.6 ± 0.7
	5.9 ± 3.6[§]	4.4 ± 1.3[§]
Nicotine in the skin (μg cm^{-2})[^]	1.89 ± 1.36	2.52 ± 1.27
	nd	nd

[§]after rinsing procedure
[^]at the end of experiment
nd (not detected): < LOD

Highlights

- skin contamination by e-liquids containing nicotine at 1,8% leads to dermal absortpion and deposition of nicotine in skin

- nicotine uptake from skin contaminated by e-liquids can lead to minor health ilness in vapers

- caution must be paid to dermal contamination by e liquids in children

- skin cleaning reduces transdermal penetration and dermal accumulation of nicotine