Percutaneous metals absorption following exposure to road dust powder

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ABSTRACT

The skin constitutes a protective barrier to external physical and chemical aggressions. Although it is constantly exposed to various xenobiotics, it is generally considered poorly permeable to them, as for example metal ions, becoming unfortunately an entry route of such substances. Metals may penetrate inside the skin inducing more or less local effects such as skin sensitization and potential metals diffusion into the bloodstream. The objective of the study was to investigate the percutaneous penetration of metals *in vitro - ex vivo* in Franz cell with intact as well damaged skin applying a road dust powder. Moreover, porcine and human skins were compared. This study demonstrated that, after the application of a road dust powder on the skin, metals can penetrate and permeate this cutaneous membrane. From this experimental analysis, in intact skin lead (Pb) achieved the highest skin absorption in both human and porcine skin, while skin absorption profile of cobalt (Co) was the lowest in human skin than the one in porcine model. The concentrations of Ni present in receiving solution were higher compared to other metals in all experiments performed. The present work, definitely shows that metals permeation through damaged skin is accelerated than intact skin, as a result of the weaker cutaneous barrier function. According to published data, pig skin appeared as a suitable model for human skin. Our results confirmed that skin absorption of metals can be relevant in environmental exposures.

1. Introduction

In occupational settings, skin is potentially exposed to chemicals, metals and other contaminants causing concern as a potential health hazard. Cutaneous contamination of metals can occur via direct contact with metallic surfaces, and other metal materials as tools, or clothing or by deposition on skin surface of airborne metal containing particles (Schneider et al., 1999); (McDougal et Boeniger, 2002). *Stratum corneum*, the outermost skin layer is often considered an effective barrier for metals absorption as it functions as a reservoir. Available bibliography reporting skin absorption of metals is not extensive. Several factors such as pH, temperature, and the presence of salts, amino acids, proteins, and skin surface lipids may affect dermal absorption (Girod, Ramotowski, et Weyermann, 2012); (Taylor et Machado-Moreira, 2013) (Hostynek, 2003); (Anja Franken et al., 2015). Percutaneous penetration of metals is closely related to the ability to form complexes with or oxidize metal atoms by the sweat (Julander et al., 2013); (Erfani, Lidén, et Midander, 2015). As being skin-thickness and concentration-dependent the metallic ions released can penetrate through the skin causing allergy or other skin disorders occurring in a short and long time period (Ward et al., 2019). However, although chromium (Cr), cobalt (Co) and nickel (Ni) are considered as trace nutrients, they are recognized as the most common cause of allergic contact dermatitis (ACD), and rarely allergic contact urticaria (Ahlström et al., 2019). They are among the so-called "skin sensitisers" because even at low doses, may induce skin allergy. It is estimated that 10–15% of women and 3% of men are affected by nickel allergy, whereas only 1–3% are cobalt and chromium-allergic (Thyssen et Menné, 2010). Once these sensitizing metals Ni, Co and Cr penetrated through the skin, they may exert some damages. For example, they interact directly with the specific histidine residues in the human Toll-like receptor 4 (TLR4). This interaction causes the activation of the intracellular pro inflammatory signalling pathways by mimicking pathogen associated molecular patterns such as TNFα, IL-8 and IL-6 (Schmidt et Goebeler, 2015). In addition, nickel and chromium (VI) may activate the cytotoxic responses of T-cell due to their bind to the major histocompatibility molecules

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(MHC) (Schmidt et Goebeler, 2015). Moreover, skin absorption of metals can be an object of investigation in various fields. Cutaneous penetration of pollutants as lead (Pb), or contact "skin sensitisers" (ex. Ni, Cr, Co), is studied for toxicological purposes, in order to help establishing decontamination protocols in case of accidental exposure (Larese et al., 2007); (Filon et al., 2004). The aim of this work is to study the percutaneous absorption of cadmium (Cd), cobalt (Co), chromium (Cr), manganese (Mn), nickel (Ni), vanadium (V), lead (Pb) and zinc (Zn) following exposure to road dust powder. Road dust is one of the main contributors to pollution and its exposure represents a common scenario of contact with pollutants. It is estimated that about 37% of particulate air pollution is due to the resuspension of this powder (Amato et al., 2009). The road dust is generated from brake and tire wear, combustion byproducts of engine oil and fly ash from asphalt (Berger et al., 2011). The elemental composition includes volatile or semi volatile organic compounds and heavy metals such as Zn, Cu, Pb, Ni, Cr and Cd from vehicular traffic and Fe, Al, and Mn from soils (Koh et Kim, 2019); (Gunawardana et al., 2012). Therefore, the toxicity of road dust is highly associated with its chemical composition as reported from several studies (Bilenko et al., 2015). Specifically, the certified reference material BCR®-723 provided by the European Institute for Reference Materials and Measurements was used as road dust formulation. The skin penetration of metals was evaluated *in vitro* in Franz cells using intact skin as well as damaged skin to estimate the effect of skin lesions. In addition, porcine and human skin were compared as skin models. Experiments were followed over 24 h and the metal concentrations in each skin layer was assessed at the end of the experience.

2. Material and methods

2.1. Chemicals

All chemicals were analytical graded: urea, sodium chloride, sodium hydrogenphosphate, potassium dihydrogenphosphate were purchased from Carlo Erba (Milan, Italy); ammonium hydroxide (25% w/v) was bought from J. T. Baker (Deventer, Holland); lactic acid (90% v/v) from Acros Organics (Geel, Belgium); nitric acid (67–69% v/v) from VWR (Milan, Italy). Water reagent grade was produced with a Millipore purification pack system (MilliQ water). The buffered physiological saline solution used as the receptor fluid was prepared by dissolving 2.38 g of Na_2HPO_4, 0.19 g of KH_2PO_4 and 9 g of NaCl into 1 L of MilliQ water (final pH = 7.35). The synthetic sweat solution used as the donor fluid consisted in 0.5% w/v sodium chloride, 0.1% w/v urea and 0.1% w/v lactic acid in MilliQ water; pH was adjusted with ammonium hydroxide (1 N) to pH 4.5 and 6.5. The indicative mass values of the elements containing in the certified reference material BCR® – 723 were listed below: Cd 2.5 mg/kg; Co 29.8 mg/kg; Cr 440 mg/kg; Mn 1.28 g/kg; Ni 171 mg/kg; Pb 866 mg/kg; V 74.9 mg/kg; Zn 1.66 g/kg.

2.2. Dissolution test of road dust powder (BCR®-723)

To evaluate the extent of ionization of metals from the road dust powder (BCR®-723), the dissolution of powder in synthetic sweat solutions and in buffered physiological saline solution was carried out. Briefly, 0.50 g of BCR®-723 were added into 10 mL of synthetic sweat solutions at two different pH (pH 4.5 and 6.5) and in 10 mL of buffer solution pH 7.35. Periodic quantification of metals concentration was monitored and analyzed via Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES). At different time points (1 h, 8 h, 24 h), 1.0 mL of each suspension was collected, added into 10 mL of MilliQ water, and filtered through a nylon membrane disc filter (0.22 µm poresize) before ICP – OES analysis. The experiment was performed in triplicate.

2.3. Preparation of road dust powder (BCR®-723) in synthetic sweat solution

The road dust powder solution was freshly prepared by weighing 0.50 g of BCR®-723 in 10 mL of synthetic sweat at pH 4.5. Before application in the donor chamber, the suspension was sonicated in an ultrasonic bath for 10 min in order to disperse the powder as homogeneously as possible.

2.4. SKIN samples preparation

2.4.1. Porcine SKIN membranes
Piglet-ears were collected immediately after animals were killed. They were stored at −25 °C on parafilm until use for a maximum period of 4 months. Porcine skin was used as a model of human skin in the penetration test due to its similarity in terms of morphology and permeability to human skin (Schmook, Meingassner, et Billich, 2001); (Barbero et Frasch, 2009); (Wester et al., 1998). On the day of the experiment, the piglet-ears were thawed in physiological solution at room temperature and the skin samples were cut into 4 cm^2 square pieces. The thickness was measured with a micrometer (Mitutoyo). The thicknesses of piglet-ear skin membranes were 0.90 ± 0.2 mm. For the damaged skin we used an abraded skin protocol as suggested by Bronaugh and Steward (Bronaugh et al., 1985): skin was abraded by drawing the point of a 19-gauge hypodermic needle across the surface (6 marks in one direction and 6 perpendicular). To evaluate skin integrity, Trans Epidermal Water Loss (TEWL) was measured on each skin piece using a Vapometer (Delfin Vapometer, Delfin Technologies, Sweeden). The cut-off for acceptable TEWL was within the range of 10 g m^{-2}·h^{-1} (Guth et al., 2015). Samples having TEWL values above 10 g m^{-2}·h^{-1} after 1 min were discarded.

2.4.2. Human SKIN membranes
Human abdominal flank full thickness skin was obtained as surgical waste approved by the Trieste Hospital Ethical Committee n° 236/2007. Donors were men and women with a range of age from 45 to 71 years. Prior to storage in a freezer (−25 °C), subcutaneous fat was removed with a scalpel blade and the hair was shaved from the epidermis. Skin samples were stored in a freezer at −25 °C for a period of up to 4 months. On the day of the experiment, skin samples were thawed in physiological solution at room temperature and the skin samples were cut into square sections of 4 cm^2. Skin samples were prepared to the final thickness of 1.05 ± 0.02 mm (Micrometer Mitutoyo). A damaged skin sample was obtained according to the method described above (section 2.4.1.). Skin integrity was checked by measuring the Trans Epidermal Water Loss (TEWL) (Delfin Vapometer, Delfin Technologies, Sweeden) and was found to be below 10 g m^{-2}·h^{-1} (Guth et al., 2015).

2.5. In vitro permeation and metals distribution IN SKIN layers after 24 H exposure

Skin absorption studies were performed in static diffusion cells according to OECD guidelines (OECD, 2004). The skin pieces were mounted between the donor and receptor chamber of Franz-type static diffusion cells with the *stratum corneum* facing the donor chamber. The effective skin area of diffusion was 0.95 cm^2. The receptor fluid (RF) was composed of physiological solution continuously stirred using a Teflon coated magnetic stirrer. The concentration of the salt in the acceptor fluid is approximately the same that can be found in the blood. The receptor compartment had a mean volume of 4.5 mL filled with RF. Mounted Franz cells were maintained at 32 ± 1 °C by means of circulation of thermostated water in the jacket surrounding the cell. The protocol for testing the skin permeation of metals after exposure to road dust was derived from the protocol described by Filon et al. (FilonLarese et al., 2009). The skin experiments were carried out as follows:

Exp. 1 pig-ears skin: Briefly, infinite doses of 1.0 mL of pure freshly

made suspension of road dust (5%w/v of BCR® −723) in synthetic sweat at pH 4.5 was applied on the skin surface. As BCR® −723 is a multi-elements powder with a range of different metal concentrations, the quantity of 1.0 mL in the donor compartment was chosen in order to get infinite doses for all tested metals. This resulted in an applied dose of Q_0 = 52,6 mg/cm^2. The donor compartment was closed with parafilm during the time of the experiment. The permeation study was then carried out for 24 h, in order to determine the amount of each metal remaining in the skin. At each run, 3 cells were carried out. The experiment was performed twice with a total of 6 independent cells.

Exp. 2 pig-ears damaged skin: Experiences were carried on according to the method described above (Exp. 1) using an abraded skin protocol as suggested by Steward (Bronaugh et al., 1985) (see section 2.4.1).

Exp. 3 human abdominal skin: Experiments were performed following the same procedure described above (Exp. 1) but replacing the porcine skin by human abdominal skin. At each run, 3 cells were carried out. The experiment was performed twice with a total of 6 independent cells.

Exp. 4 human abdominal damaged skin: Experiences were carried on according to the method described above (Exp. 3) using an abraded skin protocol as suggested by Steward (Bronaugh et al., 1985) (see section 2.4.1).

Controls: A skin sample with no road dust powder applied on the skin surface was used as control at each run. The experiment was performed twice with a total of 8 independent cells. The donor chamber was filled with synthetic sweat at pH 4.5 and the manipulation was performed as described for skin absorption studies (Exp.1).

The amounts of metals eluted to RF and recovered inside the skin layers after 24 h were quantified by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS).

2.6. Collection and treatment of samples

After 24 h exposure, the cells were dismantled. All the acceptor fluid was removed, and frozen for the following analysis. The non-absorbed fraction was removed from the skin surface by washing the donor chamber thrice with 1.0 mL of MilliQ water for 20 s and gentle wiped with a cotton swab. After, skin layers were separated as follows:

For Exp. 1 and 2. The *stratum corneum* (SC) was isolated from viable layers by tape stripping (18 strips) using D-Square tape (Monaderm) and placed in different tubes each containing 10 mL of HNO$_3$ 69% v/v; 5.0 mL of MilliQ water. The tubes were heated at 70 °C for 60 min (PerkinElmer Touch Controller). Separation allows the determination of metals in the *stratum corneum* (SC). SC fractions were previously diluted 1:50 in MilliQ water before ICP-MS analysis. Then, the skin fractions were collected and stored in freezer at −25 °C before digestion treatment (see section 2.7).

For Exp. 3 and 4. The viable epidermis (VE) was separated from the dermis (D) by heat treatment (1 min in water at 60 °C) before digestion of the tissue (see section 2.7).

Receptor fluid was previously diluted 1:10 in MilliQ water acidified with 1% nitric acid before ICP-MS analysis.

2.7. SKIN digestion after the experiment

At the time of the analysis, the skin membranes were thawed and the exposed area was weighted placed in Teflon based sealed beaker with 2.0 mL of HNO$_3$ 69% v/v; 0.50 mL of H$_2$O$_2$; 1.0 mL of MilliQ water. Afterward, the reaction mixture was heated in a microwave oven (Multiwave-PRO, Anton Paar) at 180 °C for 25 min. After the digestion treatment, the solutions were diluted 1:10 in MilliQ water for the ICP-MS analysis.

2.8. Analytical measurements

2.8.1. Quantification analysis by inductively coupled plasma –optical emission spectroscopy (ICP – OES)

Total metal (Cd, Co, Cr, Mn, Ni, Pb, V and Zn) concentrations in the solutions resulting from the dissolution experiences were performed by Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP – OES) using an Optima 8000 ICP – OES Spectrometer (PerkinElmer, USA) equipped with an S10 Autosampler (PerkinElmer, USA). The analyses were conducted using a calibration curve obtained by dilution (range: 0–10 mg L^{-1}) of a multistandard solution (10 mg L^{-1}) for ICP analyses (Periodic Table MIX 1, Sigma-Aldrich). The precision of the measurements as relative standard deviation (RSD%) for the analysis was always less than 5%.

2.8.2. Quantification analysis by inductively coupled plasma-mass spectrometry (ICP-MS)

Metal contents (Co, Cr, Ni and Pb) of controls and road dust formulation-exposed skin samples together with acceptor solutions were evaluated by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) using a NexION 350x Spectrometer (PerkinElmer, USA) equipped with an ESI SC Autosampler. The analysis was performed in KED mode (Kinetic Energy Discrimination) using ultra-high purity helium (flow rate of 4.8 mL min^{-1}) to control and minimize cell-formed polyatomic ion interference. Instrument calibration was carried out using standard solutions (linear in the concentration range of 0.1–100 µg L^{-1}, R^2 = 0.999) according to the dilution of a multistandard solution 10 mg L^{-1} for ICP analysis (Instrument Calibration Standard 1, PerkinElmer, USA). The measurements of samples were performed using the calibration curve method obtained by analyzing standard solutions for instrumental calibration. The Limits of Detection (LOD) for each element are listed below: Co 0.0002 µg L^{-1}; Cr 0.003 µg L^{-1}; Ni 0.005 µg L^{-1}; and Pb 0.0009 µg L^{-1}. The coefficients of variation of repeatability (RSD %) were <3%.

2.9. Statistical analysis

Results were expressed as quantity penetrated per skin surface unit (ng·cm^{-2}). Data from skin absorption experiments were expressed as mean ± standard deviation (SD). The differences between independent data were evaluated by the nonparametric Mann-Whitney test. Significance level was set at p < 0.05. Data were treated and analyzed with Excel for Windows (release 2010) and Stata Software (version 11.0; StataCorp LP, College Station, TX, USA).

3. Results

3.1. Solubility profile of metals from the road dust powder in synthetic sweat and in buffer solutions

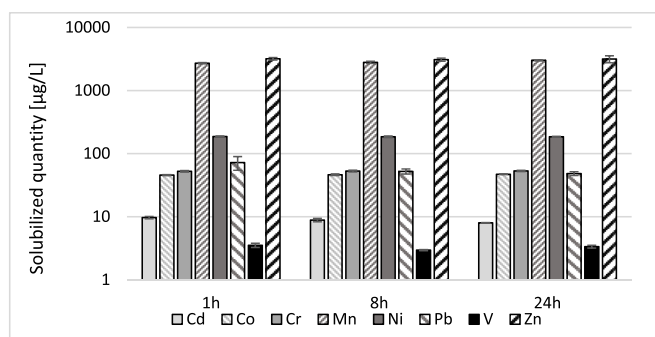The dissolution study was performed to evaluate the metal solubility

Fig. 1. Solubility profile of metals in synthetic sweat at pH 4.5.

profiles in synthetic sweat at different pH and in buffer solution. Figs. 1–3 report the concentration of solubilized metals over time in synthetic sweat at pH 4.5, 6.5 and in buffered physiological saline solution pH 7.35, expressed in absolute values (µg/L). Also, the metals ion percentage, displayed in Table 1, is obtained by the ratio between the steady state metal ion concentrations (listed in Figs. 1–3) and mass values of each metals containing in certified reference material BCR® – 723. The results demonstrated that almost all metals were less soluble in buffer solution compared to synthetic sweat solutions (Figs. 1–3). Only V showed higher solubility in saline solution than synthetic sweat solutions (around 13 µg/L *vs* 3.5 µg/L). The fraction of metals ionized increased ranging between 3 and 5% for Mn and Zn (Table 1). These two metals had the highest dissolution profiles around 3000 µg/L in both synthetic sweat solutions as observed in Figs. 1 and 2. These data were expected as a consequence of their high amount in the certified reference material BCR®-723 (Mn 1.28 g/kg; Zn 1.66 g/kg). Similarly, the study of Jang et al. (Jiang and Yang, 2014) showed that Zn contained in size–segregated particulate matter (PM2.5) was one of the highest soluble element in water reaching a percentage value higher than 80%. In our study, the lowest solubility and the lowest percentage of metal ions (around 3 µg/L and 0.1% respectively) was found for V in both synthetic sweat solutions. Although, the percentage of Cd ions was higher up to 6%, this metal showed lower dissolution profile around 8 µg/L in synthetic sweat solution, due to its minimum concentration in the BCR®-723 powder (Cd 2.5 mg/kg). Contrary, Cd and V contained PM2.5 were the most water soluble metals with their solubility higher than 70% (Jiang and Yang, 2014). Concerning the present study, since Cd and V showed lower solubility profiles in synthetic sweat solutions as a function of their content in the road dust, they were not considered for the absorption test. In addition, even though Mn and Zn were higher soluble in synthetic sweat solution, they are oligoelements essential and beneficial to living beings involved in several metabolic, enzymatic and immunological processes (Carvalho et al., 2015), so they were discarded for the skin permeation test. Moreover, the three skin sensitisers (Co, Cr, Ni) have a similar dissolution behavior in both synthetic sweat solutions (Figs. 1 and 2). However, Jang et al. (Jiang and Yang, 2014) found that Cr was the lowest water soluble element (<20%) in PM2.5 and the solubility values of Pb, Mn and Ni were between 40 and 60%. Related to our results, it can be observed that after 1 h, metals reached the maximum rate of dissolving ranging 50 µg/L, showing no significant differences until the end of 24 h. The solubility of Pb was found to decrease over time in all medium tested (Figs. 1–3). The results showed no significant difference in solubility test at pH 4.5 and 6.5, thus a common profile can be observed for almost all metals in both synthetic sweat solutions. The choice of synthetic sweat solution at pH 4.5 was dictated to reproduce the workplace conditions and in agreement with our previous studies (FilonLarese et al., 2009); (Larese et al., 2007). The pH of human skin is typically in the range of 4–5.5, but in some individuals' physical activities, the pH may reach lower level, promoting the oxidation of metals to ions, and thus their absorption through the skin. The synthetic sweat solution at pH 4.5 was used as donor solution
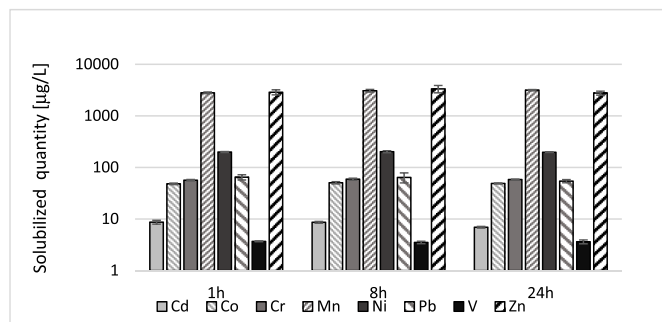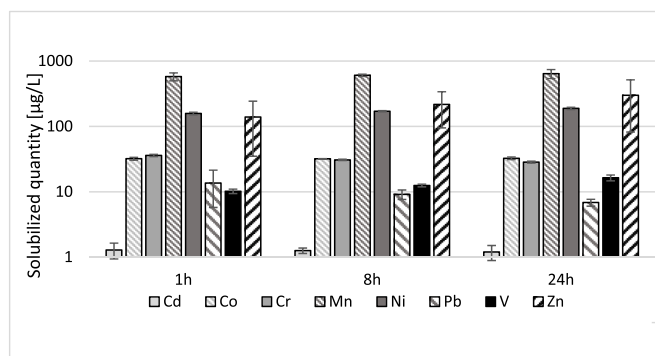

Fig. 3. Solubility profile of metals in physiological solution pH 7.35.

for the *in vitro* absorption study. In conclusion, to assess the permeation experience, nickel, cobalt and chromium as sensitizing metals were selected. Also lead was considered for the test due to its ability to produce harmful effects in humans, even at relatively low levels of exposure (Sun et al., 2002).

3.2. Road dust powder SKIN absorption

Skin absorption of the selected metals (Co, Cr, Pb and Ni) from road dust powder were investigated. 1.0 mL/cm^2 of freshly prepared road dust (5%w/v of BCR® −723) were applied on the skin surface, which corresponds to a skin deposition of 1.57 µg/cm^2 for Co, 23.1 µg/cm^2 for Cr, 9.0 µg/cm^2 for Ni and 45.5 µg/cm^2 for Pb. The study was carried out for 24 h using the Franz cell method, the gold standard for percutaneous studies (OECD, 2004). Porcine skin was used as the most relevant animal model because of its similar morphological and functional characteristics as human skin (Simon et al., 2000). Damaged skin was chosen as a model of barrier-disrupted skin as is the case in some skin pathologies (dermatitis, scratches, diseases, sunburn). Penetration in both intact and damaged skins was investigated to evaluate the metal ions behavior on skin conditions. The amounts of Co, Cr, Pb and Ni permeated in receptor fluid (RF) and their distribution throughout the skin layers after 24 h exposure are presented in Figs. 4 and 5 and Tables 2–4. The results clearly showed the metals penetration in damaged and to a lesser extent in intact skin (Table 2 and Fig. 4). The highest total amount of metals penetrated in porcine intact skin were observed for Pb: 549 ng/cm^2, Co: 518 ng/cm^2 and Ni: 353 ng/cm^2, while the lowest penetration was measured for Cr content (183 ng/cm^2) after 24 h of contact. On damaged skin, the amount penetrated was increased compared to intact skin for all tested metals (from 0.7 to 1.8%) (Fig.4), as expected due to the impairment of the barrier function (Kezic et al., 2009). Further, considering the total quantity retained in the skin, the metals ratio of blank samples was lower than exposed skin samples in intact and damaged skin. Then, Cr and Co concentrations in blank samples were close to those in intact skin samples. Statistically significant differences were difficult to obtain which were related to the variability typical for the results of skin absorption experiments.

However, to better predict the metal permeation profile through the skin, the study was also performed on human skin explants. Post-exposure, the amounts of metal that were retained in skin were quantified and shown in Fig. 5, Table 3.

As can be seen in Fig. 5, in intact human skin Pb exhibited a significantly higher skin penetration of 68.8 ng/cm^2 compared to other metals. The total quantity retained in the skin of Cr and Ni was 32.0 ng/cm^2 and 31.0 ng/cm^2 respectively. The lowest penetration in intact skin was measured for Co content (1.85 ng/cm^2). Interestingly Co permeation in porcine skin was 500-fold higher than human skin (Fig. 4). However, the penetration of metals across porcine skin was significantly higher than that of human skin. According to different studies the percutaneous absorption in common laboratory animals is higher or

Fig. 2. Solubility profile of metals in synthetic sweat at pH 6.5.

	Time	Element (%)							
		Cd	Co	Cr	Mn	Ni	Pb	V	Zn
Synthetic sweat solution pH 4.5	1 h	7.73 ± 0.34	3.06 ± 0.06	0.24 ± 0.01	4.24 ± 0.09	2.19 ± 0.04	0.17 ± 0.04	0.09 ± 0.01	3.85 ± 0.18
	8 h	7.08 ± 0.45	3.11 ± 0.09	0.24 ± 0.01	4.38 ± 0.18	2.17 ± 0.06	0.12 ± 0.01	0.08 ± 0.00	3.72 ± 0.21
	24 h	6.41 ± 0.07	3.16 ± 0.03	0.24 ± 0.01	4.70 ± 0.08	2.17 ± 0.03	0.11 ± 0.01	0.09 ± 0.00	3.80 ± 0.48
Synthetic sweat solution pH 6.5	1 h	6.93 ± 0.60	3.23 ± 0.11	0.26 ± 0.01	4.37 ± 0.17	2.33 ± 0.05	0.15 ± 0.02	0.10 ± 0.01	3.47 ± 0.39
	8 h	6.90 ± 0.26	3.38 ± 0.17	0.27 ± 0.01	4.81 ± 0.29	2.36 ± 0.11	0.15 ± 0.03	0.09 ± 0.01	4.03 ± 0.66
	24 h	5.57 ± 0.20	3.29 ± 0.07	0.26 ± 0.01	4.92 ± 0.15	2.31 ± 0.03	0.13 ± 0.01	0.10 ± 0.01	3.34 ± 0.30
Physiological saline solution pH 7.35	1 h	1.03 ± 0.28	2.15 ± 0.11	0.16 ± 0.01	0.90 ± 0.13	1.84 ± 0.08	0.03 ± 0.02	0.27 ± 0.02	0.17 ± 0.13
	8 h	1.01 ± 0.10	2.15 ± 0.01	0.14 ± 0.00	2.00 ± 0.04	2.36 ± 0.11	0.02 ± 0.00	0.33 ± 0.02	0.26 ± 0.15
	24 h	0.96 ± 0.25	2.19 ± 0.10	0.13 ± 0.00	2.21 ± 0.09	2.31 ± 0.03	0.02 ± 0.00	0.44 ± 0.04	0.36 ± 0.26

Fig. 4. Metal contents found in the cutaneous tissue after 24 h exposure in intact and damaged pig skin. Applied dose was 52.6 mg/cm^2. Data are given as mean ± SD. Stars show the statistically significant differences (p < 0.05).
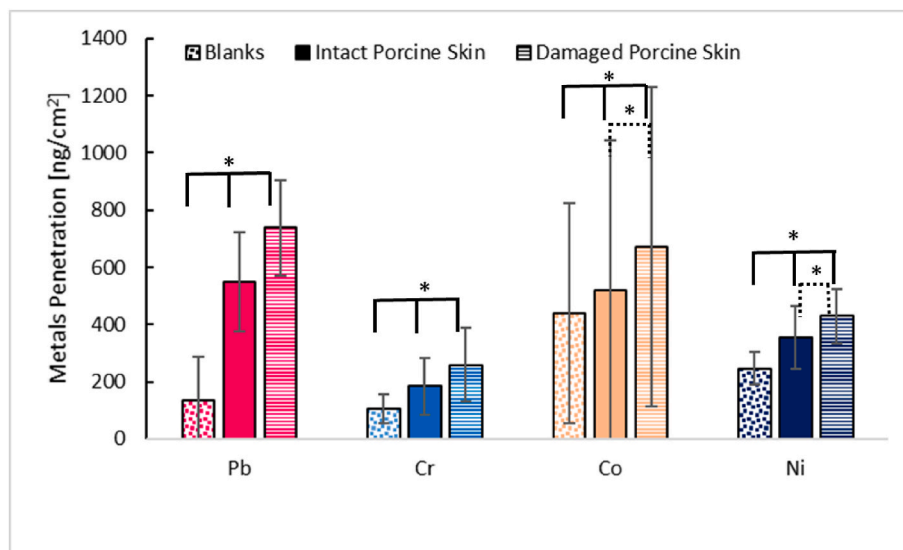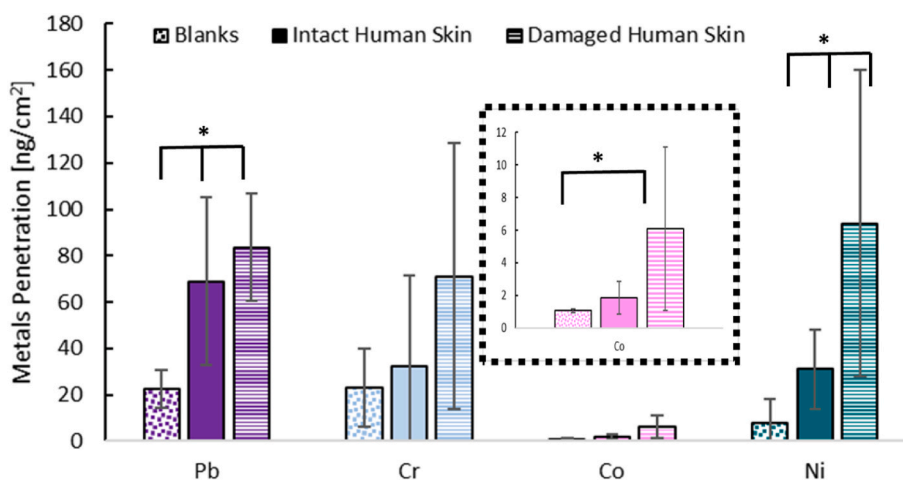

Fig. 5. Metal contents found in the cutaneous tissue after 24 h exposure in intact and damaged human skin. Applied dose was 52.6 mg/cm^2. Data are given as mean ± SD. Stars show the statistically significant differences (p < 0.05).

more closely related to human skin (Dick et Scott, 2011); (Sato, Sugibayashi, et Morimoto, 1991); (Panchagnula, Stemmer, et Ritschel, 1997). Therefore, the fat deposition and vascularisation of the cutaneous glands of porcine skin are different from human skin. As expected, metal contents found on damaged skin was enhanced due to the removal of the barrier function. Contrary to porcine skin, the highest penetration in

damaged human skin was measured for Ni content (94 ng/cm^2). It can be noticed that the total amounts of metals penetrated in the skin in blank samples differed compared to exposed samples. These results were also expected, but a more surprising result was obtained for Co, where its accumulation was similar to those in intact skin sample (1.06 ng/cm^2 vs 1.85 ng/cm^2). Further, Co, Cr, Ni and Pb concentrations in the

Table 2

Metal amounts found in skin layers in blanks, intact and damaged pig skin after 24 h exposure. Applied dose was 52.6 mg/cm^2. Data are given as mean ± SD. Asterisk (*) indicates statistically significant difference obtained between blanks and intact or damaged skin in the Mann-Whitney test (p < 0.05).

		SC (ng/cm^2)	E + D (ng/cm^2)	Total (ng/cm^2)
Pb	Blanks	117 ± 139	19.4 ± 12.9	136 ± 150
	Intact Skin	121 ± 71.3	428 ± 133	549 ± 175*
	Damaged Skin	150 ± 32.3	589 ± 140	739 ± 167
Cr	Blanks	64.5 ± 32.7	40.6 ± 19.3	105 ± 51.7
	Intact Skin	73.4 ± 51.8	110 ± 54.8	184 ± 101*
	Damaged Skin	82.0 ± 50.6	177 ± 103	259 ± 129*
Co	Blanks	439 ± 382	1.2 ± 1.2	440 ± 383
	Intact Skin	489 ± 495	29.2 ± 29.5	518 ± 524*
	Damaged Skin	634 ± 528	38.0 ± 31.7	672 ± 559*
Ni	Blanks	150 ± 27.6	95.2 ± 32.2	245 ± 57.1
	Intact Skin	102 ± 63.1	251 ± 73.8	353 ± 110*
	Damaged Skin	103 ± 33.3	325 ± 80.9	429 ± 94.4*

Table 3

Metal amounts found in skin layers in intact and damaged human skin after 24 h exposure. Applied dose was 52.6 mg/cm^2. Data are given as mean ± SD. Asterisk (*) indicates statistically significant difference obtained between blanks and intact or damaged skin using Mann-Whitney test (P < 0.05).

Metal	Blanks (ng/cm^2)	Intact Skin (ng/cm^2)	Damaged Skin (ng/cm^2)
Pb	22.5 ± 8.0	68.8 ± 36.2*	83.6 ± 23.1*
Cr	23.2 ± 16.8	32.2 ± 39	71.1 ± 57.5
Co	1.06 ± 0.1	1.85 ± 1	6.1 ± 5*
Ni	8.00 ± 10	31.0 ± 17*	94 ± 66*

receptor fluid (RF) expressed in ng/cm^2 were summarized in Table 4. The mean amounts of the selected metals reaching the RF in both skin models followed the order as follows: Ni > Cr > Pb > Co. As shown in Table 4, in intact porcine skin Cr and Pb permeation was 10-fold slower than damaged porcine skin (16.9 ng/cm^2 *vs* 26.9 ng/cm^2 and 7.7 ng/cm^2 *vs* 17.7 ng/cm^2 respectively). Similarly, in human skin Cr permeation was 10-fold slower than damaged porcine skin (10.3 ng/cm^2 *vs* 23.6 ng/cm^2). On the contrary, Pb content observed in RF across intact and damaged skin was 3.24 ng/cm^2 and 5.56 ng/cm^2 respectively. Moreover, in damaged porcine skin Ni permeation was 25-fold higher than intact skin (80.9 ng/cm^2 *vs* 55.9 ng/cm^2), indeed in human model it was 29-fold higher (48.3 ng/cm^2 *vs* 19.5 ng/cm^2). Notably, in both skin models the amount of Co was similar for intact and damaged skin. Considering blank samples, the results demonstrated that metal contents in RF were lower than exposed skin samples in intact and damaged for porcine model. Similarly, in human model the concentration of all metals in blanks were lower compared to intact and damaged skin

Table 4

Concentrations of Co, Cr, Pb and Ni (ng/cm^2) in the acceptor medium in blanks, porcine and human skin. (*) statistically significant difference obtained between blanks and intact or damaged skin in the Mann-Whitney test (¥) Statistically significant differences (p < 0.05) between intact and damaged skin. (†) Statistically significant differences (p < 0.05) between two skin models: human skin and porcine skin.

Skin Model	Pb (ng/cm^2)	Co (ng/cm^2)	Cr (ng/cm^2)	Ni (ng/cm^2)
Blanks Porcine skin	5.77 ± 1.4	0.47 ± 0.4	6.69 ± 7.6	10.87 ± ND
Intact Porcine Skin	7.72 ± 3.5	6.29 ± 2.3	16.9 ± 10.2†	55.9 ± 21.7†
Damaged Porcine Skin	17.7 ± 14.2†	6.67 ± 1.8	26.9 ± 22.1†	80.9 ± 30.5 ¥
Blanks Human skin	3.39 ± 3.7	0.59 ± 0.09	2.63 ± 0.7	8.41 ± 4.3
Intact Human Skin	3.24 ± 1.9*	0.81 ± 0.2	10.3 ± 2.3*	19.5 ± 9.2*
Damaged Human Skin	5.56 ± 2.3*	1.68 ± 0.8*	23.6 ± 13.2*	48.3 ± 22.5* ¥

samples. Surprisingly, the amounts of Pb and Co permeated in blanks were close to intact skin samples for both skin models. No significant difference was found for all metals permeated in RF through intact and damaged skin in porcine skin, contrary in human model, metal concentrations increased significantly. Statistically significant difference between porcine and human skin models were found for Pb, Cr and Ni.

Finally, the permeability coefficient K$_p$ (cm/h) of each metal ion was determined dividing the effective absorption rates by the equilibrium concentration of each metal ion in the donor solution. The obtained values were summarized in Table 5. For both skin models, the K$_p$s of Pb were higher than other metal ions with values of $3.53*10^{-1}$ and $3.98*10^{-2}$ in intact skin; $5.30*10^{-1}$ and $5.46*10^{-2}$ in damaged skin respectively. In intact and damaged porcine skin, the lowest K$_p$ was observed for Ni ranging around $0.34*10^{-1}$ and $0.57*10^{-1}$ respectively. On the other hand, in human model Co was the less permeable ion with K$_p$s measured in the range of $0.09*10^{-2}$ and $0.54*10^{-2}$ in intact and damaged skin.

4. Discussion

A series of *in vitro* experiments to measure dermal absorption of four metals from the road dust powder were assessed on Franz static diffusion cells. It is well reported that metals readily penetrate through the skin in their ionized form (A. Franken et al., 2014). In our study the skin was exposed to multiple metals contained into road dust powder. Metallic ions (e.g., nickel, cobalt, chromium, lead) may influence the percutaneous absorption, since they are able to diffuse through this cutaneous membrane. Most elements increase their ionized form as acidity increases: in some case it becomes approximately 10 to 100-fold higher at each one pH unit decreases (Zlotogorski, 1987). For this reason, our experiments were performed using a synthetic sweat solution at pH 4.5 in order to reproduce the typically pH of the skin around 4 to 5.5 which can also be lower in some cases and facilitate the permeation of metals. Our results demonstrated that metals albeit in small amounts permeate through the skin compared to the concentration in the donor compartment. The amount of metals reaching the receptor fluid was higher for Ni, and Cr, and lower for Co and Pb either in human or porcine skin. Concerning human skin samples exposed to road dust suspension, it is important to point out that the concentration of Pb and Co found in the receptor medium were comparable to those observed in blank samples. Specifically, the values obtained at 24 h were respectively: 3.39 ng/cm^2 in intact human skin *vs* 3.24 ng/cm^2 in blanks for Pb; and 0.81 ng/cm^2 in intact human skin *vs* 0.59 ng/cm^2 in blanks for Co. So, permeation of these metals through the skin was negligible. On the other hand, the permeation of Cr and Ni in exposed samples was orders of magnitude higher than the levels measured in blanks (Cr: 10.3 ng/cm^2 in intact human skin *vs* 2.62 ng/cm^2 in blanks; Ni: 19.5 ng/cm^2 in intact human skin *vs* 8.41 ng/cm^2 in blanks). In line with this study, Midander et al. (Midander and Schenk, 2020) observed a greater percutaneous permeation of nickel (0.023 μmol/cm^2) compared to Cr (0.0005 μmol/cm^2) and Co (0.0013 μmol/cm^2) in pig skin after 2 h of exposure. Moreover, the study of Larese Filon et al. (FilonLarese et al., 2006) showed a permeation of lead oxide around 2.9 ng/cm^2 through

Table 5

Skin permeability coefficients (K$_p$s) for each metal ions.

Permeability coefficient K$_p$ (cm/h)		Porcine Model	Human Model
Pb	Intact Skin	$3.53*10^{-1}$	$3.98*10^{-2}$
	Damaged Skin	$5.30*10^{-1}$	$5.46*10^{-2}$
Cr	Intact Skin	$0.69*10^{-1}$	$1.31*10^{-2}$
	Damaged Skin	$1.37*10^{-1}$	$5.39*10^{-2}$
Co	Intact Skin	$0.75*10^{-1}$	$0.09*10^{-2}$
	Damaged Skin	$2.12*10^{-1}$	$0.54*10^{-2}$
Ni	Intact Skin	$0.34*10^{-1}$	$0.76*10^{-2}$
	Damaged Skin	$0.57*10^{-1}$	$2.84*10^{-2}$

intact human skin. In fact, a potential explanation of these findings could be attributed to the different ion concentration found in the donor phases applied on the skin. Several research studies investigated permeation of metals using metal salts (Fullerton et al., 1986); (Samitz and Katz, 1967); (Florence and Lilley, 1988); (Tanojo et al., 2001); (Hostýnek, 2003) or metal/oxide nanoparticles (Mauro et al., 2015); (Crosera et al., 2016); (Larese Filon et al., 2013). The percutaneous diffusion of nickel ions from a chloride solution ($NiCl_2$) through full-thickness human skin was found to be 50 times faster than nickel ions from a sulfate solution ($NiSO_4$) (Fullerton et al., 1986). Similarly, the study of Samitz et al. (Samitz and Katz, 1967) showed that Cr (III) nitrate marginally permeated through the skin compared to Cr(III) sulfate, which was impenetrable (Samitz and Katz, 1967). These results demonstrate the specific interplay of the counter ion on the permeation rate. Furthermore, also the study of Crosera et al., (2016) concerning metal NPs confirms the capability of the skin to accumulate nickel ions. From the study of Crosera (Crosera et al., 2016), it was visible that nanoparticles may be retained in skin (9.67 ± 2.70 µg/cm^2), but metal ions were found in the receptor compartment (0.032 ± 0.010 µg/cm^2) representing higher potential to reach the systemic circulation. As it can be noticed from our data, penetration of metals into intact skin differs from non-intact skin. Percutaneous absorption was much more pronounced in damaged skin as a consequence of the less efficient cutaneous barrier function due to histological and skin microenvironment changes. In fact, wounds, scratches, inflammation, disorders of the lipid composition and organization as in the case of atopic dermatitis (Elias et al., 2008) or alteration of the epidermal differentiation in case of psoriasis, ichthyosis, skin cancer (Griffiths et al., 2007); (Marukian et al., 2016) alter the skin barrier properties. However, the evaluation of percutaneous absorption in a skin model close to the skin pathology is often underestimated and a key effect to consider, since penetration can be thoroughly different than in intact skin (Kezic et al., 2009); (Chiang and Tudela, 2012). Globally, the total amounts of metals found in skin layers and RF in blank samples were lower compared to exposed skin samples, which was an expected result. Considering the quantity penetrated in intact skin samples, higher amount was reported for Pb in both skin models, while lowest concentrations of Co were found on human skin compared to porcine skin. Moreover, the amount of Cr and Ni retained in human intact skin was relatively equal, whereas Cr concentration was lower compared to Ni in porcine skin. K_p for each metal was also determined in order to compare the percutaneous kinetics. This parameter describes the membrane penetration and as can be noticed from our data Pb was the highest permeable ion in both skin models. The lowest K_ps were measured for Ni and Co in porcine and human model respectively. Although K_p is the most convenient parameter, the published data concerning the metals percutaneous absorption are scattered and limited, so it seems difficult to make a comparison. Our K_p values can be collected in order to gradually increase the database. Therefore, our data related to pig model are in agreement with the study of Midander and Schenk, 2020). The authors demonstrated that the skin content of Co, Cr, and Ni was consistently higher in the combined exposure compared to a single metal exposure in porcine model (3.269 µg/cm^2 vs 2.394 µg/cm^2 for Co; 3.603 µg/cm^2 vs 2.967 µg/cm^2 for Cr; 3.678 µg/cm^2 vs 2.526 µg/cm^2 for Ni). Furthermore, in the study of Filon et al. (FilonLarese et al., 2009) the skin was exposed to a single exposure of each metal powders and the authors found higher accumulation of Co and Ni (29.6 µg/cm^2 and 82.3 µg/cm^2 respectively) in the human intact skin compared to Cr (14.4 µg/cm^2). The results obtained from the current study concerning human model differ from the study of Filon et al. (FilonLarese et al., 2009) because percutaneous absorption of metals in single exposure varies from those in combined exposure. These differences may be due to the fact that multiple metals influence their permeation and their extent retained in skin. However, our data concerning the amount of Co, Cr, Ni and Pb reaching the receptor compartment is the same order of magnitude as (FilonLarese et al., 2009) and (FilonLarese et al., 2006). Thus, exposure to road dust containing multiple metals leads a systemic absorption which is equal to a single metal exposure, even though the applied dose in the present experiment is 1000 fold lower (µg/cm^2 vs mg/cm^2). This work successfully identified potential health hazard of road dust, showing effects even at relatively lower dose. The simultaneous detection of multiple metals is an attractive feature to take in consideration in order to improve the understanding of risks associated with metals skin exposure and to develop new preventive measures. As expected, the absorption on damaged skin was accelerated due to the weaker cutaneous barrier function. Concerning the different absorption profiles, a probable explanation may be due to the electrophilic nature of many metals which determines their skin proteins binding capacity, resulting in depot formation in the *stratum corneum* (Samitz et al., 1976). This binding may occur in all layers of the skin to the extent of creating a secondary barrier, reducing the metals diffusion (Hostynek, 2003). Also, Franken et al. demonstrated that low permeability of metals through human skin is attributed to the valence state of the metal and to metal binding to skin components (Anja Franken et al., 2015). Furthermore, it can be also supposed that these skin absorption differences could be due to the skin model. In fact, concerning organic compounds, it is generally known that skin from adult pigs and piglets showed positive correlations to human skin, providing the most suitable experimental model for dermatological research (Schenk et al., 2018); (Barbero et Frasch, 2009); (Schmook, Meingassner, et Billich, 2001); (Hawkins et Reifenrath, 1986). Nevertheless, knowledge on the suitability of frozen pig skin as a model for human skin regarding metals absorption is still lacking. It is important to point out that the current study has some limitations. In first place, it is performed in static Franz cells, an *in vitro* method, which may not reproduce the real scenario. The obtained results can underestimate the *in vivo* conditions, because only passive diffusion is evaluated while in *in vivo* condition skin absorption can be enhanced by active mechanisms. However, to mimic sweat the *stratum corneum* was exposed for 24 h, but the excessive hydration can promote the absorption of many compounds. Further the over hydration represents a drawback of the Franz cell test in any case if it is performed with either a low or a high amount of solution in the donor compartment.

5. Conclusion

The present study aimed at investigating metals absorption in two different skin models. It has been demonstrated that in applied conditions, penetration of metals will be limited in intact skin, compared to damaged skin. The results demonstrated that the highest skin penetration (metals' content into the skin) was observed for Pb in intact human and porcine skin tissues, while skin permeation (metals pass across the skin reaching receptor compartment) was higher for Ni in all models tested. In porcine model lower concentration of Cr was retained in the skin than other metals, whereas Co showed the lowest penetration profile in human skin. This can be explained by the probable stronger binding capacity of metals to skin proteins and by the differences of these two skin tissues. Therefore, the permeability of metals in porcine skin was qualitatively similar to human skin but quantitatively different. Overall, pig provides a suitable experimental model for human skin. To conclude, the increase of penetration capacity accordingly to the pathological state of the skin is a key factor to consider, since it is able to promote absorption of such compounds. Our study suggests that skin can be a route of entry of metals after contact with environmental pollutants such as road dusts. The role of the skin in environmental pollution is generally neglected, however small amount of metals can be permeated in the skin, mainly if impaired. This exposure could be relevant for sensitizing metals, such as nickel, for which we demonstrated the higher permeation potential. More studies are needed to confirm the role of environmental exposure to metals' powders for nickel allergy.

Credit author contribution statement

Greta Camilla Magnano: Investigation, Data Curation, Writing - original draft. **Giovanna Marussi**: Investigation. **Elena Pavoni**: Investigation. **Gianpiero Adami**: Supervision. **Francesca Larese Filon**: Conceptualization, Funding acquisition, Supervision, Writing - review & editing. **Matteo Crosera**: Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Ahlström, Malin G., Thyssen, Jacob P., Wennervaldt, Michael, Menné, Torkil, Johansen, et Jeanne D., 2019. Nickel allergy and allergic contact dermatitis: a clinical review of immunology, epidemiology, exposure, and treatment. Contact Dermatitis 81 (4), 227–241. https://doi.org/10.1111/cod.13327.

Amato, F., Pandolfi, M., Escrig, A., Querol, X., Alastuey, A., Pey, J., Perez, N., Hopke, et P.K., 2009. Quantifying road dust resuspension in urban environment by multilinear engine: a comparison with PMF2. Atmos. Environ. 43 (17), 2770–2780. https://doi.org/10.1016/j.atmosenv.2009.02.039.

Barbero, Ana M., Frederick Frasch, et H., 2009. Pig and Guinea pig skin as surrogates for human in vitro penetration studies: a quantitative review. Toxicol. Vitro 23 (1), 1–13. https://doi.org/10.1016/j.tiv.2008.10.008.

Berger, Janne, et Bruce, Denby, 2011. A generalised model for traffic induced road dust emissions. Model description and evaluation. Atmos. Environ. 45 (22), 3692–3703. https://doi.org/10.1016/j.atmosenv.2011.04.021.

Bilenko, Natalya, Brunekreef, Bert, Beelen, Rob, Eeftens, Marloes, de Hoogh, Kees, Hoek, Gerard, Koppelman, Gerard H., Wang, Meng, van Rossem, Lenie, Gehring, et Ulrike, 2015. Associations between particulate matter composition and childhood blood pressure — the PIAMA study. Environ. Int. 84 (novembre), 1–6. https://doi.org/10.1016/j.envint.2015.07.010.

Bronaugh, Robert L., Stewart, et Raymond F., 1985. Methods for in vitro percutaneous absorption studies V: permeation through damaged skin. J. Pharmaceut. Sci. 74 (10), 1062–1066. https://doi.org/10.1002/jps.2600741008.

Carvalho, Dayene C., Coelho, Luciana M., Soledad, Maria, Acevedo, M.S.F., Coelho, et Nívia M.M., 2015. The oligoelements. In: Handbook of Mineral Elements in Food, édité par Miguel de la Guardia et Salvador Garrigues, vols. 109–22. John Wiley & Sons, Ltd, Chichester, UK. https://doi.org/10.1002/9781118654316.ch5.

Chiang, Audris, Tudela, Emilie, Maibach, Howard I., et al., 2012. Percutaneous absorption in diseased skin: an overview: percutaneous absorption in diseased skin. J. Appl. Toxicol. 32 (8), 537–563. https://doi.org/10.1002/jat.1773.

Crosera, Matteo, Adami, Gianpiero, Mauro, Marcella, Bovenzi, Massimo, Baracchini, Elena, Larese Filon, et Francesca, 2016. In vitro dermal penetration of nickel nanoparticles. Chemosphere 145 (février), 301–306. https://doi.org/10.1016/j.chemosphere.2015.11.076.

Dick, Ian P., Scott, et Robert C., 2011. Pig ear skin as an in-vitro model for human skin permeability. J. Pharm. Pharmacol. 44 (8), 640–645. https://doi.org/10.1111/j.2042-7158.1992.tb05485.x.

Elias, Peter M., Steinhoff, et Martin, 2008. Outside-to-Inside" (and now back to "outside") pathogenic mechanisms in atopic dermatitis. J. Invest. Dermatol. 128 (5), 1067–1070. https://doi.org/10.1038/jid.2008.88.

Erfani, Behnaz, Lidén, Carola, Midander, et Klara, 2015. Short and frequent skin contact with nickel: short and frequent SKIN contact with nickel. Contact Dermatitis 73 (4), 222–230. https://doi.org/10.1111/cod.12426.

Filon, Larese, Francesca, Maina, Giovanni, Adami, Gianpiero, Venier, Marta, Coceani, Nicoletta, Bussani, Rossana, Massiccio, Marilena, Barbieri, Pierluigi, Spinelli, et Paolo, 2004. In vitro percutaneous absorption of cobalt. Int. Arch. Occup. Environ. Health 77 (2), 85–89. https://doi.org/10.1007/s00420-003-0455-4.

Filon, Larese, Francesca, Crosera, Matteo, Timeus, Elisa, Adami, Gianpiero, Bovenzi, Massimo, Ponti, Jessica, Maina, et Giovanni, 2013. Human skin penetration of cobalt nanoparticles through intact and damaged skin. Toxicol. Vitro 27 (1), 121–127. https://doi.org/10.1016/j.tiv.2012.09.007.

Francesca Larese, Filon, Boeniger, Mark, Maina, Giovanni, Adami, Gianpiero, Spinelli, Paolo, Damian, et Adriano, 2006. Skin absorption of inorganic lead (PbO) and the effect of skin cleansers. J. Occup. Environ. Med. 48 (7), 692–699. https://doi.org/10.1097/01.jom.0000214474.61563.1c.

Francesca Larese, Filon, D'Agostin, Flavia, Crosera, Matteo, Adami, Gianpiero, Bovenzi, Massimo, Maina, et Giovanni, 2009. In vitro absorption of metal powders through intact and damaged human skin. Toxicol. Vitro 23 (4), 574–579. https://doi.org/10.1016/j.tiv.2009.01.015.

Florence, T.M., Lilley, S.G., Stauber, et J.L., 1988. SKIN absorption OF lead. The Lancet 332 (8603), 157–158. https://doi.org/10.1016/S0140-6736(88)90702-7.

Franken, A., Eloff, F.C., Du Plessis, J., Badenhorst, C.J., Jordaan, A., Du Plessis, et J.L., 2014. In vitro permeation of platinum and rhodium through caucasian skin. Toxicol. Vitro 28 (8), 1396–1401. https://doi.org/10.1016/j.tiv.2014.07.007.

Franken, Anja, Eloff, Frederik C., Du Plessis, Jeanetta, Du Plessis, et Johannes L., 2015. In vitro permeation of metals through human skin: a review and recommendations. Chem. Res. Toxicol. 28 (12), 2237–2249. https://doi.org/10.1021/acs.chemrestox.5b00421.

Fullerton, Ann, Andersen, Jan Rud, Hoelgaard, Annie, Menné, et Torkil, 1986. Permeation of nickel salts through human skin in vitro. Contact Dermatitis 15 (3), 173–177. https://doi.org/10.1111/j.1600-0536.1986.tb01320.x.

Girod, Aline, Ramotowski, Robert, Weyermann, et Céline, 2012. Composition of fingermark residue: a qualitative and quantitative review. Forensic Sci. Int. 223 (1–3), 10–24. https://doi.org/10.1016/j.forsciint.2012.05.018.

Griffiths, Christopher EM., Nwn Barker, Jonathan, 2007. Pathogenesis and clinical features of psoriasis. The Lancet 370 (9583), 263–271. https://doi.org/10.1016/S0140-6736(07)61128-3.

Gunawardana, Chandima, Goonetilleke, Ashantha, Egodawatta, Prasanna, Dawes, Les, Kokot, et Serge, 2012. Source characterisation of road dust based on chemical and mineralogical composition. Chemosphere 87 (2), 163–170. https://doi.org/10.1016/j.chemosphere.2011.12.012.

Guth, Katharina, Schäfer-Korting, Monika, Fabian, Eric, Landsiedel, Robert, Ravenzwaay, et Ben van, 2015. Suitability of skin integrity tests for dermal absorption studies in vitro. Toxicol. Vitro 29 (1), 113–123. https://doi.org/10.1016/j.tiv.2014.09.007.

Hawkins, George S., Reifenrath, et William G., 1986. Influence of skin source, penetration cell fluid, and, partition coefficient on in vitro skin penetration. J. Pharmaceut. Sci. 75 (4), 378–381. https://doi.org/10.1002/jps.2600750411.

Hostýnek, J.J., 2003. Flux of a nickel(II) salt versus a nickel(II) soap across human skin in vitro. Exog. Dermatol. 2 (4), 216–222. https://doi.org/10.1159/000076805.

Hostynek, J.J., 2003. Factors determining percutaneous metal absorption. Food Chem. Toxicol. 41 (3), 327–345. https://doi.org/10.1016/S0278-6915(02)00257-0.

Jiang, Sabrina Y.N., Fenhuan Yang, Ka Lok Chan, Ning, et Zhi, 2014. Water solubility of metals in coarse PM and PM 2.5 in typical urban environment in Hong Kong. Atmos. Pollut. Res. 5 (2), 236–244. https://doi.org/10.5094/APR.2014.029.

Julander, Anneli, Midander, Klara, Herting, Gunilla, Thyssen, Jacob P., White, Ian R., Wallinder, Inger Odnevall, Lidén, et Carola, 2013. New UK nickel-plated steel coins constitute an increased allergy and eczema risk: new UK COINS and the nickel allergy risk. Contact Dermatitis 68 (6), 323–330. https://doi.org/10.1111/cod.12092.

Kezic, Sanja, Nielsen, et J.B., 2009. Absorption of chemicals through compromised skin. Int. Arch. Occup. Environ. Health 82 (6), 677–688. https://doi.org/10.1007/s00420-009-0405-x.

Koh, Byumseok, Kim, et Eun-Ah, 2019. Comparative analysis of urban road dust compositions in relation to their potential human health impacts. Environ. Pollut. 255 (décembre), 113156. https://doi.org/10.1016/j.envpol.2019.113156.

Larese, Francesca, Gianpietro, Adami, Venier, Marta, Maina, Giovanni, Renzi, et Nadia, 2007. In vitro percutaneous absorption of metal compounds. Toxicol. Lett. 170 (1), 49–56. https://doi.org/10.1016/j.toxlet.2007.02.009.

Marukian, Nareh V., Choate, et Keith A., 2016. Recent advances in understanding ichthyosis pathogenesis. F1000Research 5 (juin), 1497. https://doi.org/10.12688/f1000research.8584.1.

Mauro, Marcella, Crosera, Matteo, Pelin, Marco, Florio, Chiara, Bellomo, Francesca, Adami, Gianpiero, Apostoli, Piero, et al., 2015. Cobalt oxide nanoparticles: behavior towards intact and impaired human skin and keratinocytes toxicity. Int. J. Environ. Res. Publ. Health 12 (7), 8263–8280. https://doi.org/10.3390/ijerph120708263.

McDougal, James N., Boeniger, et Mark F., 2002. Methods for assessing risks of dermal exposures in the workplace. Crit. Rev. Toxicol. 32 (4), 291–327. https://doi.org/10.1080/20024091064255.

Midander, Klara, Schenk, Linda, Julander, et Anneli, 2020. A novel approach to monitor skin permeation of metals in vitro. Regul. Toxicol. Pharmacol. 115 (août), 104693. https://doi.org/10.1016/j.yrtph.2020.104693.

OECD, 2004. Guideline for the Testing of Chemicals: Skin Absorption: in Vitro Method (N°428).

Panchagnula, R., Stemmer, K., Ritschel, et W.A., 1997. Animal models for transdermal drug delivery. Methods Find. Exp. Clin. Pharmacol. 19 (5), 335–341.

Samitz, M.H., Katz, Sidney, Shrager, et Joseph D., 1967. Studies of the diffusion of chromium compounds through Skin**From the section of industrial dermatology, department of dermatology, university of Pennsylvania, school of medicine, philadelphia, Pa. 19104. J. Invest. Dermatol. 48 (6), 514–520. https://doi.org/10.1038/jid.1967.83.

Samitz, M.H., Katz, S.A., et al., 1976. Nickel-epidermal interactions: diffusion and binding. Environ. Res. 11 (1), 34–39. https://doi.org/10.1016/0013-9351(76)90108-0.

Sato, Kiyoshi, Sugibayashi, Kenji, Morimoto, et Yasunori, 1991. Species differences in percutaneous absorption of nicorandil. J. Pharmaceut. Sci. 80 (2), 104–107. https://doi.org/10.1002/jps.2600800203.

Schenk, Linda, Rauma, Matias, Fransson, Martin N., Johanson, et Gunnar, 2018. « percutaneous absorption of thirty-eight organic solvents in vitro using pig skin ». Édité par dimitris fatouros. PLoS One 13 (10), e0205458. https://doi.org/10.1371/journal.pone.0205458.

Schmidt, Marc, Goebeler, et Matthias, 2015. Immunology of metal allergies: review. JDDG J. der Deutschen Dermatol. Gesellschaft 13 (7), 653–659. https://doi.org/10.1111/ddg.12673.

Schmook, Fritz P., Meingassner, Josef G., Billich, et Andreas, 2001. Comparison of human skin or epidermis models with human and animal skin in in-vitro percutaneous absorption. Int. J. Pharm. 215 (1–2), 51–56. https://doi.org/10.1016/S0378-5173(00)00665-7.

Schneider, T., Vermeulen, R., Brouwer, D.H., Cherrie, J.W., Kromhout, H., Fogh, et C.L., 1999. Conceptual model for assessment of dermal exposure. Occup. Environ. Med. 56 (11), 765–773. https://doi.org/10.1136/oem.56.11.765.

Simon, Gad A., Maibach, et Howard I., 2000. The pig as an experimental animal model of percutaneous permeation in man: qualitative and quantitative observations – an overview. Skin Pharmacol. Physiol. 13 (5), 229–234. https://doi.org/10.1159/000029928.

Sun, Chee-Ching, Wong, Ten-Tsao, Hwang, Yaw-Huei, Chao, Kun-Yu, Jee, Shiou-Hwa, Wang, et Jung-Der, 2002. Percutaneous absorption of inorganic lead compounds. AIHA J. 63 (5), 641–646. https://doi.org/10.1080/15428110208984751.

Tanojo, Hanafi, Hostýnek, Jurij J., Mountford, H. Scott, Maibach, et Howard I., 2001. In vitro permeation of nickel salts through human stratum corneum. Acta Derm. Venereol. 81, 19–23. https://doi.org/10.1080/000155501753279596.

Taylor, Nigel AS., Machado-Moreira, et Christiano A., 2013. Regional variations in transepidermal water Loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans. Extreme Physiol. Med. 2 (1) https://doi.org/10.1186/2046-7648-2-4.

Thyssen, Jacob P., Menné, et Torkil, 2010. Metal allergy—a review on exposures, penetration, genetics, prevalence, and clinical implications. Chem. Res. Toxicol. 23 (2), 309–318. https://doi.org/10.1021/tx9002726.

Ward, J.P., Franks, S.J., Tindall, M.J., King, J.R., Curtis, A., Evans, et G.S., 2019. Mathematical modelling of contact dermatitis from nickel and chromium. J. Math. Biol. 79 (2), 595–630. https://doi.org/10.1007/s00285-019-01371-2.

Wester, Ronald C., Joseph, Melendres, Sedik, Lena, Howard, Maibach, Riviere, et Jim E., 1998. Percutaneous absorption of salicylic acid, theophylline, 2,4-dimethylamine, diethyl hexyl phthalic acid, andp-aminobenzoic acid in the isolated perfused porcine skin flap compared to manin vivo. Toxicol. Appl. Pharmacol. 151 (1), 159–165. https://doi.org/10.1006/taap.1998.8434.

Zlotogorski, A., 1987. Distribution of skin surface PH on the forehead and cheek of adults. Arch. Dermatol. Res. 279 (6), 398–401. https://doi.org/10.1007/BF00412626.