Assessment of dermal absorption of beryllium and copper contained in temple tips of eyeglasses

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ARTICLE INFO

Keywords:
Beryllium
Cutaneous exposure
Dermal absorption
Franz cell
Porcine skin
in-vitro study

ABSTRACT

Dermal exposure to hazardous substances such as chemicals, toxics, metallic items and other contaminants may present substantial danger for health. Beryllium (Be) is a hazardous metal, especially when inhaled and/or in direct contact with the skin, associated with chronic beryllium disease (CBD) and Be sensitization (BeS). The objective of this study was to investigate the percutaneous penetration of beryllium and copper contained in metallic items as eyeglass temple tips (specifically BrushCAST® Copper Beryllium Casting Alloys containing Be 0.35 < 2.85%; Cu 95.3–98.7%), using Franz diffusion cells. This work demonstrated that the total skin absorption of Cu was higher (8.86%) compared to Be (4.89%), which was expected based on the high percentage of Cu contained in the eyeglass temple tips. However, Be accumulated significantly in the epidermis and dermis (up to 0.461 μg/cm^2) and, to a lesser extent, in the *stratum corneum* (up to 0.130 μg/cm^2) with a flux of permeation of 3.52 ± 4.5 μg/cm^2/hour and lag time of 2.3 ± 1.3 h, after cutaneous exposure of temple tip into 1.0 mL artificial sweat for 24 h. Our study highlights the importance of avoiding the use of Be alloys in items following long-term skin contact.

1. Introduction

In environmental and occupational settings, the skin is an important route of entry for many substances. Although inhalation is considered the most current exposure pathway, the cutaneous exposure is a big concern and one of the most probable risks associated with hazardous agents. Notably, the outermost skin layer, stratum corneum, made of dead keratinized cells embedded in a mixture of organized lipids assembled as “brick and mortar” (Elias, 1983), forms a remarkable barrier for permeation of xenobiotics in general, such as chemicals, metals,etc,but it may also be an entry gate for these molecules. Skin absorption of a substance through intact skin depends on many factors such as the physicochemical form of the compound, vehicle (aqueous, organic, particle), pH, exposure time, contaminated area, and the presence and type of wounds (Nielsen et al., 2016); (Guy, 2010); (Hostynek, 2003); (Roberts et al. 2008). Contrary, in case of wounded skin, the transcutaneous passage of a molecule is enhanced due to the disruption of the protective layer, the stratum corneum (Kezic and Nielsen, 2009) (Nielsen, 2005). Metals in nano dimensions penetrate and permeate the skin in higher amounts (Larese Filon et al. 2016); (Brouwer et al. 2016) compared to bulk materials due to their smaller dimensions (Larese Filon et al. 2015). Therefore, percutaneous permeation of metals may activate localized immune responses in allergic individuals, ultimately resulting in allergic contact dermatitis (ACD) and systemic intoxication owing to the potential metal diffusion into the circulatory system. It is know that percutaneous penetration of metals is closely related to the ability of sweat to form complexes with or oxidize metal atoms (Julander et al. 2013); (Erfani et al., 2015). Beryllium (Be) is a metal having specific and unique physicochemical properties such as light weight, high melting temperature, extreme stiffness, non-corrosiveness, and nonsparking, making it essential for medical diagnostics, nuclear/fusion reactors, and aerospace applications (Taylor et al. 2003). The main effects associated with Be exposure are sensitization (BeS) and berylliosis also known as chronic Be disease (CBD) (MacMurdo et al. 2020); (Saltini and Amicosante, 2001); (Sharma et al., 2010). Copper is an essential trace element (ETEs) providing beneficial effects in skin growth and activity, but its potential to induce skin irritation reactions remains controversial (Hostynek and Maibach, 2004).

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Accepted 8 April 2022

Exposure to Cu is commonly manifested due to its presence in such routine objects such as jewelry, coins and tools. However, direct and prolonged skin contact with copper may lead to electrochemical reactions resulting in copper ions being released which are able to diffuse through the SC in order to reach the epidermis, becoming locally or systemically available (Hostynek, 2003). It has been demonstrated that copper ions can cause toxicity in humans such as ROS production, DNA strand breaks and base oxidation (Kawanishi et al., 1989). Moreover, the excess copper may induce peroxidative damage to membrane lipids, reduce the activity of cytochrome c oxidase and impair mitochondrial respiration (Gaetke, 2003). Furthermore, knowledge regarding dermal irritation by copper and its compounds is not extensive and the role of copper as an irritant/sensitizer has been reported without providing a proper conclusion. A recent study suggested that Cu may be considered a weak sensitizer compared to other metallic compounds (Fage et al., 2014). According to the International Agency for Research on Cancer (IARC) Be and Be compounds are category 1 carcinogens and carcinogenic (Mulware, 2013), while Cu is not classifiable as to its carcinogenicity to humans. Avenues for Be exposure prevention of exposed workers include respiratory protective devices and protective clothing. Immunosuppressive therapies, such as corticosteroids are currently used to reduce CBD, but no cure is available for this lung disorder (Sizar and Talati, 2021). The aim of this work is to study the dermal absorption of Be and Cu, specifically BrushCAST® Copper Beryllium Casting Alloys (Be 0.35 < 2.85%; Cu 95.3–98.7%), following exposure to eyeglass temple tips. In the current work, cutaneous penetration of Be and Cu was investigated *in vitro* using static Franz diffusion cells. Porcine skin has been used as the most suitable experimental model for dermatological research due to its positive correlations to human skin (Schenk et al. 2018); (Barbero and Frederick Frasch, 2009); (Schmook et al., 2001); (Hawkins and Reifenrath, 1986). Experiments were performed for 24 h and the metal concentrations in each skin layer were assessed at the end of the experiment.

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). Reagent grade water was obtained using a Millipore purification pack system (MilliQ water). The physiological 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 liter 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. BrushCAST® Copper Beryllium Casting Alloys (Be 0.35 < 2.85%; Cu 95.3–98.7%).

2.2. Dissolution tests of beryllium and copper from the eyeglass bridge

To assess the extent of ionization of Be and Cu from the eyeglass bridge, the dissolution of the bridge in synthetic sweat solutions was carried out. Specifically, BrushCAST® Copper Beryllium Casting Alloys Be 0.35 - < 2.85%; Cu 95.3–98.7% was tested. Briefly, an eyeglass bridge with a surface area of 4.16 cm^2, was added into 10 mL of synthetic sweat solutions at different pH values (pH 4.5 and 6.5). The periodic quantification of Be and Cu concentrations was monitored and analyzed *via* Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP – OES) as described below in "Analytical measurement" (paragraph 2.7.1). At scheduled time points (4 h, 8 h, 24 h, 48 h, 72 h, 5 days, 7 days, 15 days, and 30 days), 0.5 mL aliquots of the artificial sweat containing eyeglass bridge were withdrawn, diluted in 5.0 mL of MilliQ water and analyzed by ICP – OES. An equal volume of fresh artificial sweat was replaced at each time interval. The experiment was performed in triplicate.

2.3. Skin samples preparation

2.3.1. 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 because of its similarity in terms of morphology and permeability to human skin (Schmook et al., 2001); (Barbero and Frederick Frasc, 2009); (Wester et al. 1998); (Simon and Maibach, 2000). On the day of the experiment, the piglet ears were thawed in a physiological solution at room temperature, and the skin samples were cut into 4 cm^2 square pieces. The thickness was measured using a micrometer (Mitutoyo). The thickness of pig ear skin membranes was < 0.97 ± 0.03 mm.

To evaluate skin integrity, Trans Epidermal Water Loss (TEWL) was measured on each skin piece after one hour of equilibration using a vapometer (Delfin Vapometer, Delfin Technologies, Sweden). The cut-off for acceptable TEWL was within the range of 10 $g{\cdot}m^{-2}{\cdot}h^{-1}$ (Guth et al. 2015). Samples having TEWL values above 10 $g{\cdot}m^{-2}{\cdot}h^{-1}$ after 1 min were discarded.

2.4. In vitro permeation and distribution in skin layers after 24h 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 salt in the receptor fluid is approximately as the same that 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 skin absorption experiments were carried out as follows:

2.4.1. Exp. 1

Briefly, the eyeglass temple tip with a surface area of 0.53 cm^2, was deposited in direct contact with the skin surface in the Franz cell with 1.0 mL of synthetic sweat at pH 4.5. The temple tip was made of the same Be-Cu alloy of the eyeglass bridge used for the dissolution test (paragraph 2.2.), and it was left on the cutaneous surface for the entire duration of the experiment (24 h). The amount of 1.0 mL of synthetic sweat at pH 4.5 in the donor compartment was chosen to completely cover the eyeglass temple tip. The donor compartment was closed with parafilm during the experiment. The permeation study was then carried out for 24 h to determine the permeation profile of Be and Cu remaining and permeating through the skin. At selected time points (4 h, 8 h, 12 h, 20 h, 24 h) 1.5 mL of each receptor sample was collected and analyzed. An equal volume of fresh receptor fluid was immediately replaced in each sample. In each run, 5 cells were carried out. The experiment was performed twice with a total of 10 independent cells. Skin from at least 4 different piglets were tested.

2.4.2. Controls

A skin sample with no temple of eyeglasses applied to the skin surface was used as a control in each run. 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 experiment was performed twice with a total of 4 independent cells. Skin from at least 4 different piglets were tested.

The amounts of Be and Cu in the RF as well as in each skin layer after 24 h were quantified by Inductively Coupled Plasma – Mass Spectrometry (ICP – MS), method described below, paragraph 2.7.1.

In an infinite dose experiment, the permeant concentration is constant over a long enough experimental duration to establish a steady state characterized by flux J reaching a constant (*i.e.*, steady state) value, designated J_{ss}. The ratio of J_{ss} (*i.e.*, the slope of the linear portion of M(t) to the external concentration difference ΔC_{ext}) is defined as the permeability coefficient, K_p. The intercept of the linear (*i.e.*, steady state) portion of M(t) with the time axis is defined as the time lag, t_L (Hopf et al. 2020).

2.5. Collection and treatment of samples

After 24 h of exposure, the cells were dismantled. All the receptor fluid was removed, and frozen for subsequent analysis. The non-absorbed fraction was removed from the skin surface by washing the donor chamber thrice with 1.0 mL of MilliQ water and wiped with a cotton swab. The temple tips of eyeglasses were collected and transferred to an Eppendorf tube®. After, skin layers were separated as follows: the *stratum corneum* (SC) was isolated from viable layers by tape stripping (4 strips) using D-Squame tape (Monaderm) and placed in different tubes each containing 5.0 mL of HNO_3 69% v/v and 10 mL of MilliQ water for 48 h. Separation allows the determination of Be in the *stratum corneum* (SC). The SC fractions were previously diluted 1:10 in MilliQ water before ICP-MS analysis. Then, the skin fractions were collected and stored in a freezer at −25 °C before digestion treatment (see Section 2.6). The receptor fluid was diluted 1:10 in MilliQ water acidified with 1% nitric acid before ICP-MS analysis.

2.6. Skin digestion after the experiment

At the time of the analysis, the skin membranes were thawed, and the exposed area was weighed placed in Teflon based sealed beaker with 2.0 mL of HNO_3 69% v/v; 0.5 mL of H_2O_2; 1.0 mL of MilliQ water. Subsequently, 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 ICP – MS analysis.

2.7. Analytical measurements

2.7.1. Quantification analysis by inductively coupled plasma –Optical emission spectroscopy (ICP – OES)

The total Be and Cu concentrations in the solutions resulting from the dissolution experiments were detrmined 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 calibration curve was linear from 0.1 to 10 mg L^{-1} ($R^2 = 0.9999$ Be; $R^2 = 0.9999$ Cu) and five calibration points from 0 to 10 mg L^{-1} (0; 0.1; 1; 5; 10) were carried out. The quantification limits (LOQ) of Be and Cu were 0.024 mg L^{-1} and 0.001 mg L^{-1}, respectively. The precision of the measurements as relative standard deviation (RSD%) for the analysis at the operative wavelengths of 313.107 nm for Be and 327.393 nm for Cu was always less than 5%. Concerning the accuracy of the method, the analysis of laboratory-fortified samples prepared by spiking 1 or 5 mg L^{-1} (depending on the Be and Cu concentrations in the investigated samples) of Be and Cu into actual samples was performed. Two laboratory-fortified samples were used.

2.7.2. Quantification analysis by inductively coupled plasma –Mass spectrometry (ICP – MS)

Be and Cu of controls and eyeglass temple tips exposed skin samples together with receptor and donor 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. The ICP-MS calibration curve was linear ($R^2 = 0.99998$ Be; $R^2 = 0.99993$ Cu) in the concentration range of 0.5–100 μg L^{-1} according to the dilution of a multistandard solution 10 mg L^{-1} for ICP analysis (Periodic Table MIX 1, TraceCERT Sigma-Aldrich). Six calibration points from 0 to 100 μg L^{-1} (0; 0.5; 1; 5; 10; 100) were used. The measurements of samples were performed using the calibration curve method obtained by analyzing standard solutions for instrumental calibration. The limits of detection (LOD) are 0.00012 μg L^{-1} for Be 9 and 0.005 μg L^{-1} for Cu 63. The quantification limits (LOQ) of Be and Cu were 0.0023 μg L^{-1} and 0.22 μg L^{-1}, respectively. The coefficient of variation of repeatability (RSD %) was < 3%. Moreover, the analysis was performed using Sc (spike of 100 μg L^{-1}, prepared by dilution from a standard solution, Scandium Standard for ICP, TraceCERT Sigma-Aldrich) as an internal standard to minimize the potential matrix effects. An additional quality control was performed by the analysis of laboratory-fortified samples prepared by spiking 1 or 5 μg L^{-1} (depending on the Be and Cu concentrations in the investigated samples) of Be and Cu into actual samples to calculate the recovery percentage. These laboratory-fortified samples were prepared for each matrix (solutions from skin digestion, SC, receptor fluid, donor compartment) to obtain a robust method for the analysis. Acceptable recoveries from spiked samples were obtained (ranging between 90% and 110%).

2.8. Statistical analysis

The results are expressed as the quantity penetrated per skin surface unit (μg·cm^{-2}). Data from skin absorption experiments are expressed as mean ± standard deviation (SD). Differences between independent data were evaluated using the nonparametric Mann-Whitney test anddata were not normally distributed. Significance level was set at $p < 0.05$. Data were treated and analyzed using Excel for Windows (release 2010) and Stata Software (version 11.0; StataCorp LP, College Station, TX, USA).

3. Results

3.1. Beryllium and copper release profiles from the eyeglass bridge in different synthetic sweat solutions

The study was performed to determine the release process of total Be and Cu into synthetic sweat at different pH. Figs. 1 and 2 display the total amount of Be and Cu released from the eyeglass bridge over time

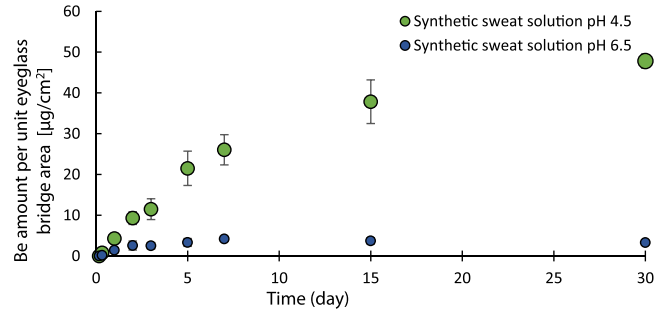

Fig. 1. Beryllium amount per unit eyeglass bridge area exposed to synthetic sweat solutions. Data expressed as mean ± SD (n = 3).

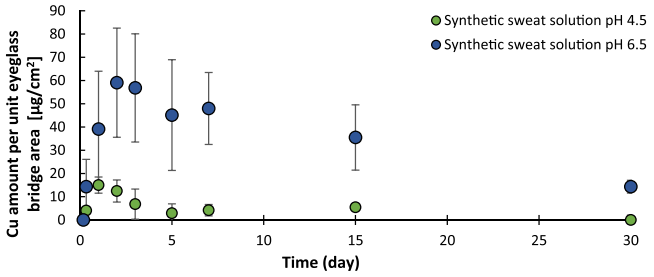

Fig. 2. Copper amount per unit eyeglass bridge area exposed to synthetic sweat solutions. Data expressed as mean ± SD (n = 3).

into synthetic sweat at pH 4.5 and 6.5. The surface area of the eyeglass bridge is 4.16 cm^2. The results, expressed as the total concentration of metals released per unit bridge area, demonstrated that both metals exhibited a time-dependent release profile. The term "per unit bridge area" represents the concentration of metal released from the eyeglass bridge in synthetic sweat solutions at different pH. In synthetic sweat at pH 6.5, Be was released reaching a plateau around 2.35 µg/cm^2, following two days without significant differences up to the end of the experiment. Contrary, the amount of Be released in synthetic sweat at pH 4.5 increased over time. The amount of Be quantified in the artificial sweat from the eyeglass bridge (per unit bridge area) was initially low, reaching 0.77 ± 0.17 µg/cm^2 after 8 h. Higher Be release was observed after 30 days of exposure: 47.2 ± 1.79 µg/cm^2 (Fig. 1). Cu showed an opposite trend in the two media investigated. Specifically, in synthetic sweat at pH 6.5 Cu was rapidly released reaching the maximum value around 59.1 ± 23.5 µg/cm^2 after two days, then it decreased achieving 14.3 ± 2.74 µg/cm^2 at the end of the experiment. Similarly, the amount of Cu quantified in synthetic sweat at pH 4.5 from the eyeglass bridge (per unit bridge area) followed a similar behavior as described for Cu in synthetic sweat at pH 6.5: Cu release was fast reaching 15 ± 3.47 µg/cm^2 during the first day of exposure, and then decreased until no further release was detected in the following 30 days (Fig. 2). A considerable decrease of Cu amounts over time could be explained by the corrosion of the alloy constituents resulting in the formation of a solid surface layer and the release of metal ions into the solution. It should be noted that the surface of the eyeglass bridge made of Be-Cu alloy is covered by a shiny reddish-brown layer after 30 days of immersion in artificial sweat. The study of Milošev and Tadeja, (2007) demonstrated that the corrosion of the Cu-18–20Zn alloy exposed to artificial sweat after 30 days, led to the formation of a solid surface layer consisting of mainly of copper oxide (Cu_2O) and copper chloride ($CuCl_2$). Moreover, it is important to point out that artificial sweat contains lactic acid and urea, which may act as complexing agents and may facilitate the dissolution process, as mentioned by (Milosev and Strehblow, 2000); (Slemnik and Milošev 2006). Therefore, we can notice that the decrease of Cu after at least 4 days of immersion in sweat, could be attributed to a precipitation process. This phenomenon may be due to carbon dioxide (CO_2) as the surface of the eyeglass bridge is covered by a greenish layer, suggesting the formation of precipitated cupric carbonate ($CuCO_3$).

3.2. Skin absorption study of beryllium and copper

3.2.1. Beryllium and copper quantification in donor solutions

Skin absorption of the selected metals from the eyeglass temple tip was investigated. The amount of Be and Cu in donor phases after 24 h of exposure was quantified using an ICP – MS. The analysis revealed that the effective dose of Be and Cu in each donor solution (DS), expressed in µg/cm^2, were 12.26 µg/cm^2 and 436 µg/cm^2 respectively.

3.2.2. Skin permeation and distribution of beryllium and copper after 24 h

The distribution throughout the skin layers after 24 h exposure to Be and Cu and their permeation in the receptor fluid (RF) are presented in

Table 1
Distribution of Be and Cu in the skin layers (SC, and E + D) from blanks and exposed skin samples after 24 h exposure. Data are given as mean ± SD (n = 10 exposed skin samples; n = 4 blank samples). Stars show the statistically significant differences obtained between blanks and exposed skin samples to Be in the Mann-Whitney test ($p < 0.05$).

		Stratum Corneum (SC) (µg/cm^2)	Epidermis (E) + Dermis (D) (µg/cm^2)	Total skin (SC+E + D) (µg/cm^2)
Be	Blanks	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.03
	Intact Skin	0.13 ± 0.09	0.46 ± 0.29	0.59 ± 0.39 *
Cu	Blanks	0.07 ± 0.02	0.13 ± 0.01	0.20 ± 0.03
	Intact Skin	0.99 ± 0.60	40.4 ± 5.66	41.4 ± 6.26 *

Table 1, Figs. 3,4, and listed in *Table 2*, Figs. 5,6 respectively. The results clearly show the total Be quantity penetrated in porcine skin samples was higher compared to blank samples, which are not exposed to Be (*Table 1*, Fig. 3). Be indicated significant accumulation in the viable epidermis and dermis (up to 0.46 µg/cm^2) and, to a lesser extent, in the stratum corneum (up to 0.13 µg/cm^2) (*Table 1*, Fig. 3). As expected, Be was detectable at very low level in the blank samples (< 0.04 µg/cm^2). Statistically significant differences between blank samples and exposed skin samples to Be were found.

As it can be seen in Fig. 4, Cu exhibited a significantly higher skin penetration of 41.4 µg/cm^2 compared to blank samples (0.20 µg/cm^2). The total quantity retained in the epidermis and dermis (E + D) and in the *stratum corneum* (SC) was 40.4 µg/cm^2 and 0.99 µg/cm^2 respectively (Table 1, Fig. 4). These results were expected due to the high percentage of Cu (95.3–98.7%) present in the eyeglass temple tips. Concerning blank samples, it can be noticed that the total amounts of Cu penetrated in all skin layers (SC, E + D) differed compared to exposed skin samples. Statistically significant differences between blank samples and Cu skin samples were found.

Further, Be and Cu concentrations in the receptor fluid (RF) expressed in ng/cm^2 are summarized in *Table 2* and in Figs. 5 and 6. The mean amounts of Be and Cu reaching the RF in exposed skin samples increased over time, reaching 38.3 ng/cm^2 and 957 ng/cm^2 respectively at the end of the contact time (24 h). The flux of Be was 3.52 ± 4.5 µg/cm^2/hour and the lag time 2.3 ± 1.3 h. Considering blank samples, the results demonstrated that both metal contents in RF were significantly lower than exposed skin samples.

Finally, the total absorption amounts of Be and Cu measured in RF+(D+E)+SC+DS were 12.89 µg/cm^2 and 478.34 µg/cm^2, respectively. Therefore, the percentage of each metal absorbed by the skin was determined by dividing the total absorption amount by the concentration of Be and Cu in each compartment and skin layer. The values

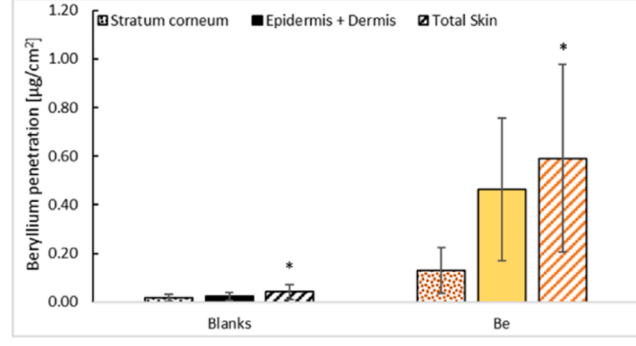

Fig. 3. Be concentration found in skin layers (SC and E + D) after 24 h exposure. Data is given as mean ± SD (n = 10 exposed skin samples; n = 4 blank samples). Stars show the statistically significant differences obtained between blanks and exposed skin samples to Be in the Mann-Whitney test ($p < 0.05$).

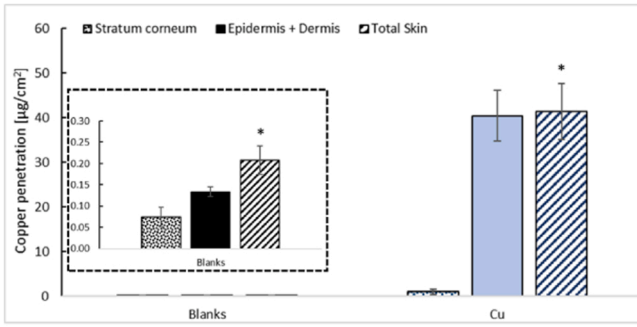

Fig. 4. Cu concentration found in skin layers (SC and E + D) after 24 h exposure. Data is given as mean ± SD (n = 10 exposed skin samples; n = 4 blank samples). Stars show the statistically significant differences obtained between blanks and exposed skin samples to Cu in the Mann-Whitney test ($p < 0.05$).

Table 2
Be and Cu concentration found in receptor fluid (RF). Values are expressed as mean ± standard error (SE) (n = 10 exposed skin samples; n = 4 blank samples).

	Blank samples (ng/cm²)		Exposed skin samples (ng/cm²)	
Time (h)	Be	Cu	Be	Cu
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
4	0.02 ± 0.02	30.9 ± 9.32	3.08 ± 1.98	31.5 ± 6.21
8	0.16 ± 0.02	40.9 ± 5.93	14.3 ± 13.0	63.0 ± 10.5
12	0.19 ± 0.15	43.5 ± 4.96	16.3 ± 14.3	76.4 ± 14.6
20	0.28 ± 0.07	46.9 ± 8.51	21.32 ± 16.8	694 ± 258
24	0.30 ± 0.00	55.3 ± 3.67	38.3 ± 20.0 *	957 ± 355 *

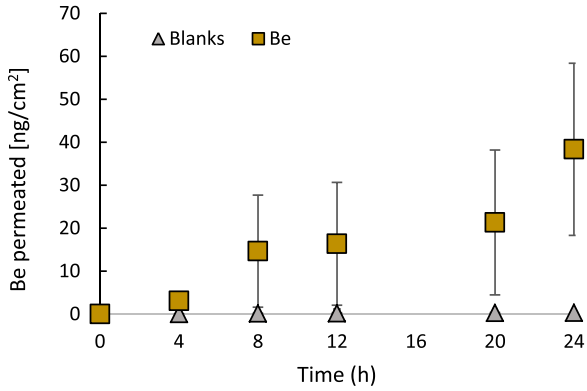

Fig. 5. Be concentration that permeated into the receptor fluid at specific extraction times. Values are expressed as mean ± standard error (SE) (n = 10 exposed skin samples; n = 4 blank samples).

obtained are summarized in Table 3.

4. Discussion

Skin absorption of Be and Cu in the eyeglass temple tips was investigated using Franz static diffusion cells. This method allows the *in vitro* measurement of molecules from the surface of a skin explant to a receptor fluid that mimics the bloodstream. It is well reported that metals readily penetrate through the skin in their ionized form (Franken et al. 2014). In the present study the skin was exposed to metal items (eyeglass temple tips) in order to simulate the skin contact with metallic surfaces where metal ions may be solubilized and absorbed into the skin. Most elements increase their ionized form as acidity increases; in some cases, it becomes approximately 10–100-fold higher at each one pH unit decreases (Zlotogorski, 1987). Further, Stefaniak et al., (2011) showed that

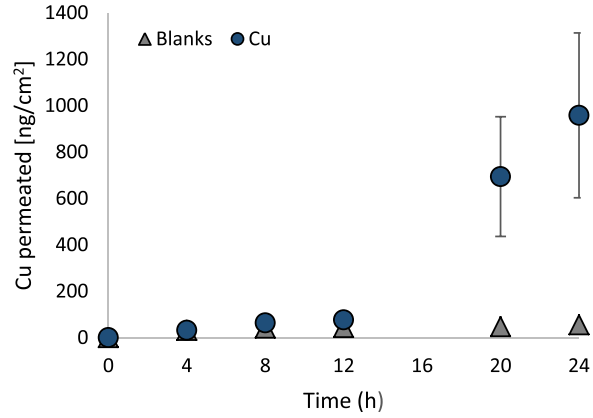

Fig. 6. Cu concentration that permeated into the receptor fluid at specific extraction times. Values are expressed as mean ± standard error (SE) (n = 10 exposed skin samples; n = 4 blank samples).

Table 3
The total absorbed amounts in RF, D, E, SC and DS of beryllium and copper. Values are expressed as percentage.

Metal	Receptor fluid (RF) %	Dermis (D) + Epidermis (E) %	Stratum corneum (SC) %	Donor solution (DS) %
Be	0.30	3.58	1.01	95.11
Cu	0.20	8.45	0.21	91.15

the rates of Be release from all reference materials tested in artificial sweat were faster at pH 5.3 than at pH 6.5. For this reason, our experiments were performed using a synthetic sweat solution at pH 4.5 in order to reproduce the typical pH of the skin around 4–5.5 which can also be lower in some cases and facilitate the permeation of metals. The preliminary tests showed the dissolution of Be and Cu from the eyeglass bridge in synthetic sweat solutions at pH 4.5 and 6.5 during 30 days. The eyeglass bridge was made of the same Be-Cu alloy used for the *in vitro* skin absorption study of eyeglass temple tips. At the initial sampling period, Be quantified in the artificial sweat at pH 4.5 per unit bridge area was low (0.77 ± 0.17 µg/cm² after 8 h), then it increased over time with a final plateau around 47.2 ± 1.79 µg/cm² after 30 days of exposure. Be released in synthetic sweat at pH 6.5 reached a plateau of 2.35 µg/cm² after two days, then no significant differences were observed up to 30 days. The amount released is relevant compared to the restriction limit of nickel (Ni) released from articles and items intended to come into direct and prolonged contact with the skin (limit ≤ 0.5 µg/cm² per week) (Ahlström et al. 2017). However, although nickel, chromium (Cr) and cobalt (Co) are considered "skin sensitisers", the release restriction limits exist only for Ni. There is current no such legislation for Co and Cr (Wang et al. 2019); (Kettelarij et al. 2014). Concerning Be, only the allowable workplace limit for surface contamination (0.03 µg/cm²) has been reported in literature (Sanderson et al. 2008), but this comparison may be misleading due to the differences of scenario. The two hands are highly exposed compared to skin contact with eyeglass parts such as bridges and temple tips. On the other hand, in synthetic sweat at pH 6.5 Cu release was faster, reaching 59.1 ± 23.5 µg/cm² after two days, then it decreased reaching 14.3 ± 2.74 µg/cm² up to the end of the experience. The release behavior of Cu in synthetic sweat at pH 4.5 was similar to that of Cu in synthetic sweat at pH 6.5: Cu was rapidly released reaching 15 ± 3.47 µg/cm² on the first day of exposure, then decreased achieving 5.45 ± 1.37 µg/cm² at the end of 15 days, followed by no further release after 30 days. This may be explained by the formation of corrosion products with different solubilities and/or their deposition on the surface, which may locally act as a barrier for Cu release (Midander et al., 2007). Metals such as Be and Cu in contact with skin tissue include

the release of metal ions that may have a certain effect on the human body (Lansdown, 1995). The results from this study confirmed that metals such as Be and Cu can permeate through the skin and the concentrations reaching the receptor fluid after 24 h of exposure were relatively higher for Cu and lower for Be in exposed skin samples (957 $\pm$ 355 ng/cm^2 *vs* 38.3 $\pm$ 20.0 ng/cm^2). These results are in line with expectations based on literature data confirming the very low absorption rate of electrolytes, especially of metal compounds (Skog and Wahlberg, 1964). Several skin absorption studies of metals were performed using metal salts or metal/oxide nanoparticles. Some authors reported that skin exposure to soluble beryllium salts (*e.g.* BeF_2, $BeCl_2$, $Be(NO_3)_2$, $BeSO_4$) may induce sensitization in animals (Zissu et al., 1996) and humans or elicit cutaneous inflammatory reactions (Curtis, 1951); (Deubner et al. 2001). Skin absorption of Be can be relevant due to its sensitization potential, representing the first step towards developing CBD (Newman et al. 2005); (Day et al. 2006). CBD has been associated with industrial exposures; and a series of cases of CBD have been reported among family members of Be factory workers and people living near Be production facilities such as refineries (Sanderson et al. 2008). Limited data simulating the scenario of topical exposure to individual Cu salts or complexes are available in literature. A research study showed that the percutaneous diffusion of Cu ions from a chloride solution ($CuCl_2$) incorporated in ointments (petrolatum formulae) through sliced human skin was found to be higher than ions from a sulphate solution ($CuSO_4$) (22.1 μg/cm^2 *vs* 4.3 μg/cm^2) (Pirot et al. 1996). Similarly, the study of Fullerton et al. (1986) revealed that Ni from $NiCl_2$ permeated 50-fold higher than Ni from $NiSO_4$ through full-thickness human skin. These data demonstrate the specific interplay of the counter ion on the permeation rate. However, Tinkle et al. (2003) demonstrated that poorly soluble beryllium oxide particles (0.5- and 1.0-μm) applied to the human skin, in conjunction with motion, can penetrate the *stratum corneum*, epidermis and dermis, inducing Be immunologic sensitization. Even though the absorption of Be through intact skin is low (but not negligible), it is well known that percutaneous passage of metals through injured skin is more pronounced as a consequence of the less efficient cutaneous barrier function due to histological and skin microenvironment changes. In fact, wounds, scratches, inflammation, disorders of lipid composition and organization as in the case of atopic dermatitis (Elias and Steinhoff, 2008) or epidermal differentiation disorders such as psoriasis, ichthyosis and skin cancer (Griffiths and Barker, 2007; Marukian and Choate, 2016) alter the skin barrier properties. However, Ivannikov et al. (1982) observed a greater permeation of $BeCl_2$ in skin with three types of wounds abrasions, cuts and entreating wounds (deep muscle trauma) after 24 h of exposure. The results demonstrated that the concentrations of $BeCl_2$ reaching the systemic circulation were 7.8% up to 11.4% for abrasions, 18.3–22.9% for cuts, and 34–38.8% for penetrating wounds. Thereforeo, the evaluation of dermal absorption in a skin model close to the skin pathology is often underestimated and is a key factor to consider (Kezic and Nelson, 2009); (Chiang et al., 2012), because penetration can be thoroughly different from that in intact skin. Considering the metal skin content, the amount of Cu found in the whole skin in exposed samples was significantly higher than Be concentration (41.4 $\pm$ 6.23 μg/cm^2 *vs* 0.59 $\pm$ 0.39 μg/cm^2). It can be noticed that the total skin absorption of Cu was higher (8.86%) than the one of Be (4.89%), which was expected based to the high percentage of Cu in the eyeglass temple tips. Moreover, the increase in Be and Cu concentrations in the whole skin may have been due to a build-up of metals in the cutaneous membrane, forming a reservoir, followed by slow diffusion through the receptor compartment. Related to the different skin penetration of Be and Cu, a probable explanation may also be attributed to metal binding to skin components such as glycolipids, phospholipids, carboxylate, phosphate groups and carbonyl groups of sn-2 phospholipid chains. This binding may occur in all layers of the skin resulting in depot formation in the *stratum corneum*, thus reducing the metals diffusion (Samitz and Katz, 1976); (Dupuis et al. 1984); (Alder et al. 1986); (Santucci et al. 1998). Additionally, their ability to permeate skin samples can be explained by the prolonged exposure time and hydration of the skin during the experiment which represents a penetration enhancer for hydrophilic substances in some parts of the uppermost layer of the cutaneous membrane (Marjukka et al., 1999).

It is generally described in literature 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 and Frederick Frasc, 2009); (Schmook et al., 2001); (Hawkins and Reifenrath, 1986). However, there is still a paucity of knowledge on the suitability of frozen pig skin as a model for human skin regarding metal absorption. Furthermore, it is important to point out that the current study has some limitations. Firstly, the percutaneous absorption was investigated 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 vivo* skin absorption can be enhanced by active mechanisms. Secondly, it is important to highlight that piglet ear skin, used as a human skin model, is more permeable than adult pig ear skin, resulting in an overestimation of skin absorption. Additionally, to mimic sweat, the *stratum corneum* was exposed for 24 h, but the excessive hydration may promote the transcutaneous passage of many compounds.

Although the skin absorption obtained was very low, a precautionary approach is due to the sensitizing potential of beryllium and its carcinogenic effect (but only for lung exposure). Therefore, we suggest avoiding the use of beryllium alloys in items that are in prolonged contact with the skin, such as eyeglasses.

5. Conclusion

The current study definitely shows that the ionic species of Be and Cu from the eyeglass temple tips permeate and penetrate the skin compared to the unexposed samples. Our experimental findings indicate that the highest skin penetration was observed for Cu in intact porcine skin tissue compared to Be skin content. These data were expected because of the high percentage of Cu contained in the eyeglass temple tips. This can also be related to the stronger binding capacity of Cu to the skin contents compared to Be. Percutaneous penetration of metals is a complex phenomenon because several factors are interrelated such as pH, oxidation state, the presence of counter ions, dose, and solubility. In conclusion, our investigations highlight that the skin may be a route of entry of metals after contact with metallic items. Dermal absorption of Be may cause skin sensitization and we demonstrated that the prolonged contact with metal item containing $0.35 < 2.85\%$ of Be determined the permeation of a small amount of this metal through intact skin.

Finally, our study confirms the need to avoid the use of Be alloys contained in items.

CRediT authorship contribution statement

Greta Camilla Magnano: Investigation, Data curation, Writing – original draft. **Giovanna Marussi**: Investigation. **Gianpiero Adami**: Supervision. **Matteo Crosera**: Conceptualization, Supervision, Writing – review & editing. **Francesca Larese Filon**: 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.

Acknowledgements

The authors acknowledge the financial support provided by Unifarco S.p.A. Santa Giustina (BL) Italy and the University of Trieste (Italy) . The

authors also thank Davide Maccan, MD for his help with the experiments.

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