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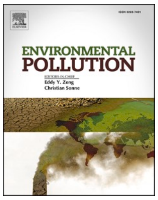

Skin absorption of metals derived from hydrogenated stainless particles in human skin: Results from the TITANS project[☆,☆☆]

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ABSTRACT

Workers involved in the decommissioning and removal of radioactive material from nuclear power plants can come into contact with tritiated dust from stainless steel. This study aimed to investigate metal penetration and permeation after skin contamination with these particles.

Static diffusion Franz cells were used with intact, damaged, or broken human skin. Stainless steel particles 316 L were applied to the donor phases, and the receiving solutions were collected at regular intervals for 24 h to determine the amount of metals that penetrated the skin. The effectiveness of the decontamination procedure was investigated after 30 min using water and soap. The metal content in the skin was evaluated after 24 h of exposure. Metals detected were Ni, Cr, Co, Mn, Cu, Mo.

For Ni, Mn, and Cu, we found a significant increase in metal permeation in all treated cells compared with the blank ($p < 0.02$). For Co and Cr, permeation through the skin was significant only in the decontaminated and broken cells ($p < 0.05$). Decontaminated skin presented higher metal permeation for Ni, Co and Cu compared to intact skin ($p < 0.05$) while broken skin presented, as expected, the higher permeation profile ($p < 0.05$) for all metals. The metal that was more represented inside the skin was Cr, with more than 15 $\mu g/cm^2$ for intact skin. Ni inside the skin reached the 10.2 ± 8.5 $\mu g/cm^2$ for intact skin.

Overall, the levels of metals in the receiving solution were very low in the case of intact and damaged skin contact, and the metal levels significantly increased only in the case of broken and decontaminated skin.

More relevant appears Skin content with sensitizing metals (Ni, Cr, and Co) that can induce allergic sensitization or cause allergic contact dermatitis in subjects already sensitized.

1. Introduction

Stainless steel particles (SSP) can be released during the decommissioning and removal of radioactive material in nuclear power plants contaminating workers. This dust can also be released into the environment. However, the effects of these powders have been poorly documented (Ferreira et al., 2023; Vernon et al., 2022; Slomberg et al., 2024), and understanding their impact on workers involved in these procedures in nuclear facilities, as well as on people living nearby, is needed. Previous studies were conducted as part of the European TRANSAT project (https://transat-h2020.eu/) (Liger et al., 2018; Ferreira et al., 2023) to study the effects of stainless steel particles, both

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hydrogenated (Vernon et al., 2022) and tritiated, to verify their impact on freshwater (Slomberg et al., 2024), rodents (Smith et al., 2022), and lung cells (Lamartiniere et al., 2022). Moreover, during the same project, a simulation of the effects on workers and populations was performed (Mentana et al., 2023a; Mentana et al., 2023b), considering the human risk associated with the inhalation of tritiated particles derived from the decommissioning of nuclear power plants.

In the ongoing European project TITANS (https://titans-project.eu/), skin exposure to tritiated SSP is considered, with the aim of fully understanding all routes of entry of powders derived from nuclear power plants into the body (Matsumoto et al., 2021). The first step in this study was to investigate the skin absorption of metals derived from hydrogenated stainless steel powder. This will help assess the amount of metals that can permeate the skin, potentially causing local and systemic effects. In the second step, experiments are performed using tritiated particles to verify the skin penetration of tritium.

Stainless steel (304 L and 316 L grades) is used in reactors, cooling systems, pumps, and containment vessels containing radioactive water or gas (Cattant et al., 2008) because of its technical properties such as high heat and corrosion resistance (Lo et al., 2009; Zinkle and Was, 2013; Fan et al., 2020).

SSP contain iron, chromium, nickel, and other metals that, if released from the particles, can cause local effects, such as allergic contact dermatitis for sensitizing metals (chromium, nickel, or cobalt), and potentially systemic effects by entering blood circulation after skin permeation (Filon et al., 2009; Taxell et al. 2020). Franz cell system permits ex vivo studies of the amount of metals that reach the skin layers (epidermis and dermis) and permeate the skin, potentially reaching systemic circulation. This approach is commonly used to study drugs adsorbed through the skin, and the Organization for Economic Co-operation and Development (OECD) has validated these methods (Hopf et al., 2020).

The aim of our work was to study the skin absorption of metals (Ni, Cr, Co, Mn, Cu, Mo) after contamination with stainless steel derived from the dismantling procedures of nuclear fusion and fission reactors. No data are available on this topic and these results are preliminary for the study of skin absorption of metals and tritium after contamination with tritiated stainless steel powders. This topic is important due to the need in future to dismantle older nuclear power plants.

2. Materials and methods

2.1. Chemicals and particles

Stainless steel particles 316 L (SSP) (Goodfellow Cambridge Ltd, UK) are spherical (based Fe with 18%Cr- 10%Ni– 3%Mo) with a mean diameter of 3 μm and were selected to correspond to three criteria: (i) a relevant composition for nuclear applications (Cattant et al., 2008, Zinkle and Was, 2013), (ii) a particle size distribution comparable to particle production during cutting operation from dismantling contaminated steel pipe (Gensdarmes et al., 2019), and (iii) a homogeneity of the shape to avoid any bias at the microscopic scale.

The protocol used for hydrogenated loading of the SSP was defined in the tritium lab in CEA Saclay (French Alternative Energies and Atomic Energy Commission) in three steps, thermally activated at 450 °C (Payet, 2020). In the first two steps, the sample was exposed to a $^{1}H_2$ atmosphere (99.9992%) at starting pressure of $1.20 \pm 0.05 \times 10^5$ Pa and $1.4 \pm 0.05 \times 10^5$ Pa, respectively. These steps allowed the surface of the particles to be controlled by heating the sample at 450 ± 5 °C for 2 h. The treatment lasted 2 h at 450 ± 5 °C and above $1.00 \pm 0.05 \times 10^5$ Pa.

The study of metal absorption after skin contact with hydrogenated particles is needed to determine absorption due to particles by themselves, which will be compared in a future study to both particulate and radiative absorption after exposure to tritiated particles.

2.2. Characterization

Characterization of SSP, chemicals used, dissolution test of stainless steel particles (AISI 316 L), preparation of stainless steel in synthetic sweat solution, preparation of Human skin membranes are reported in supplemental material n. 1.

2.3. In vitro permeation and penetration into the skin after 24h exposure

Static diffusion cells were used for skin absorption studies, following the OECD guidelines (OECD, 2004). The skin permeation of metals from hydrogenated stainless-steel particles was evaluated following the protocol used in previous studies Magnano et al., (2022), 2023.

We mounted human skin pieces between the donor and receptor chambers of Franz cells, with a skin area of 0.95 cm^2. The receiving compartment had an average volume of 4.5 mL filled with physiological solution, which was continuously stirred using a Teflon-coated magnetic stirrer. 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 L of MilliQ water (final pH = 7.35). The synthetic sweat solution used as the donor fluid consisted of 0.5% w/v sodium chloride, 0.1% w/v urea, and 0.1% w/v lactic acid in MilliQ water. The pH of two solutions of synthetic sweat was adjusted with ammonium hydroxide (1 N) to pH 4.5 and 6.5.

The salt concentration in the receptor fluid was approximately the same as that in blood. The system temperature was set at 32 ± 1 °C by circulating thermostatic water in the jacket around the cell.

2.4. Sampling

Exp. 1 intact skin: 1.0 mL of a pure freshly made suspension of stainless steel (1% w/v) in synthetic sweat at pH 4.5 were applied to the skin surface. The protocol was derived from our previous study on road dust (Magnano et al., 2022). Parafilm was used to seal the donor compartment during the experiments. The study was conducted for 24 h to study the permeation profiles of metals inside the skin and in the receiving solution. Receiving solution samples (0.5 mL) were collected at 1, 4, 8, and 24h, and then analyzed. An equal volume of fresh receiving solution was then added to each sample.

Exp. 2 damaged skin: we follow the protocol described in Exp. 1 with abraded skin using the method suggested by Steward (Bronaugh et al., 1985) and reported in supplemental material 1.

Exp. 3 decontaminated skin: samples were decontaminated using the methodology described in supplemental material 1.

Exp. 4 broken skin: in these experiments, the skin flap was pierced perpendicularly with a scalpel blade causing a 5 mm long cut in the center of the exposed area.

Blanks: A skin sample without stainless-steel powder was used as a blank in each experiment. Synthetic sweat, 1.0 mL of synthetic sweat (pH = 4.5) was added to the donor chamber, and the experiment was performed following the procedure described in Exp. 1.

Four independent biological replicates, using skin from two donors, were performed for a total of 20 samples x 4 time points (total 80 samples) and 20 skins of which 12 epidermis and 12 dermis (total 32 samples).

Metals in the receptor fluid and in the skin layers after 24 h were quantified by Inductively Coupled Plasma – Mass Spectrometry (ICP – MS), following the method described in section 2.7.

2.5. Samples collection and analysis

The cells were dismounted after 24 h of analysis, and the receptor fluid was removed and frozen for ICP-MS analysis. At the time of analysis, it was necessary to dilute the solutions 1:10 with MilliQ water and acidify them to 1% with HNO_3. The skin surface was cleaned using 1.0 mL of MilliQ water for three times to remove the unabsorbed donor phase. The skin pieces were cut circularly to obtain only the “exposed area” (0.95 cm^2), and then were separated into viable epidermis (VE) and dermis (D) by heat treatment (1 min in water at 60 °C) prior to tissue digestion (see section 2.11). The receptor fluid was diluted 1:10 in MilliQ water acidified with 1% nitric acid before the ICP-MS analysis.

2.6. *Skin digestion after experiments*

The skin membranes were weighed. Then, they were transferred into Teflon-sealed vessels with 3.0 mL of HNO_3 69% v/v, 0.5 mL of H_2O_2, and 1.0 mL of MilliQ water. The reaction mixture was heated in a microwave oven (Multiwave-PRO; Anton Paar) at 180 °C for 20 min. After digestion was completed, the contents of the vessels were transferred to 50 mL vials, brought to a volume of 20 mL with MilliQ water, and stored in a refrigerator. Before ICP-MS analysis, the solutions were diluted 1:10 in Milli-Q water.

2.7. *Analytical measurements*

The metal contents of the control, stainless-steel exposed skin samples, and donor and receiving solutions were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) with a NexION 350X spectrometer (PerkinElmer, USA) equipped with an ESI SC autosampler. For the stainless steel particles, Co, Cr, Cu, Mn, Mo, and Ni were analyzed and reported. Pb, V, As and Cd were measured but concentrations of these metals were below the instrument’s detection limits. The analysis was performed in Kinetic Energy Discrimination mode using ultra-high-purity helium at a flow rate of 4.8 mL min^{-1} to control polyatomic ion interference. The ICP-MS calibration curve was linear ($R^2 = 0.999$; selected ion mass: 60 u.m.a.) in the concentration range of 0.2–100 μg L^{-1} according to the dilution of a 10 mg L^{-1} multistandard solution for ICP analysis (Periodic Table MIX 5, TraceCERT Sigma-Aldrich). Seven calibration points from 0 to 100 μg L^{-1} (0, 0.2, 0.5, 1, 5, 10, 100) were used. The samples were analyzed using a calibration curve obtained using standard solutions.

Limits of Detection (LOD) were 0.01 μg L^{-1} for Co; 0.01 μg L^{-1} for Cr; 0.03 μg L^{-1} for Cu; 0.04 μg L^{-1} for Mn; 0.06 μg L^{-1} for Mo; and 0.04 μg L^{-1} for Ni. The coefficient of variation of repeatability (RSD %) were <3%. Therefore, the analysis was performed using Sc (45 μm. a.; peak of 200 μg L^{-1}, prepared by dilution from a 1000 mg L^{-1} Scandium Standard for ICP, TraceCERT Sigma-Aldrich) and Y (89 μm. a.; peak of 200 μg L^{-1}, prepared by dilution from a 1000 mg L^{-1} Yttrium Standard for ICP, TraceCERT Sigma-Aldrich) as internal standards to minimize potential matrix effects.

2.8. *Statistical analysis*

Data were processed using Excel for Windows (version 2010) and Stata software (version 17.0; StataCorp LP, College Station, TX, USA) to perform statistical analysis.

The results were expressed as the quantity that penetrated the skin per surface unit (μg cm^{-2}) or as the quantity that permeated per skin per surface unit (ng cm^{-2}). Differences between groups were analyzed using the Mann-Whitney *U* test. The level of significance was set at $P < 0.05$.

3. Results

Table 1 reports results of dissolution of metals from stainless steel powders using synthetic sweat at 4.5 pH to mimic the skin physiologic pH. Mainly Mn, followed by Cu and Ni were the metals with the higher dissolution potential, reaching a concentration in donor solution at T24 of 18.4 ± 3.3, 2.1 ± 0.2 and 1.6 ± 0.6 μg/cm^2, respectively. Lower amounts of other metals were released to obtain a concentration around or below 0.2 μg/cm^2.

Table 1

Results of dissolution test of stainless steel particles in synthetic sweat at pH 4.5 expressed as μg of metal released per gram of powder (n = 3; standard deviations are not reported but they varied in the range 8–20 % of the media value). In the last line the amount of metals ions released in synthetic sweat has been converted mass per unit skin surface (μg/cm^2) to calculated the cumulative effective dose.

Time (h)	Ni	Co	Cr	Cu	Mo	Mn
1h (μg/g)	111	1.1	23.1	191	5.4	1828
4h (μg/g)	133	1.4	21.1	200	6.5	1873
8h (μg/g)	165	1.7	23.0	240	8.6	2117
24h (μg/g)	149	1.8	18.8	196	7.6	1750
24h (μg/L)	1494	17.5	188	1958	76	17,502
Effective dose applied on the cells at 24 h (μg/cm^2)	1.6 ± 0.3	0.02 ± 0.00	0.2 ± 0.04	2.1 ± 0.2	0.1 ± 0.01	18.4 ± 3.3

The permeation profiles are shown in Fig. 1 and Table 2. For Ni, Mn, and Cu, we found a significant increase in metal permeation in all treated cells compared with the blank ($p < 0.02$). For Co and Cr, permeation through the skin was significant only in the decontaminated and broken cells ($p < 0.05$). For Mo, damaged, decontaminated, and broken skin showed a significant increase in metal permeation ($p < 0.05$). Damaged skin was more permeable to Ni, Mn, and Mo than intact skin ($p < 0.02$). Decontaminated skin presented higher metal permeation for Ni, Co and Cu compared to intact skin ($p < 0.05$) while broken skin presented, as expected, the higher permeation profile ($p < 0.05$) for all metals. The permeation profiles are shown in Fig. 1. For Ni, an increase in the Ni content in the receiving solution started at 1 h for decontaminated skin, reaching the highest values at 24 h (332 ± 231 ng/cm^2/24h), with values similar to those of broken skin (255 ± 26.7 ng/cm^2/24h). Intact and damaged skin presented similar profiles, but damaged skin showed an increase in Ni permeation after 1 h of exposure, while intact skin Ni permeation increased slowly, reaching similar values at 8 h. Co skin permeation was very low and significantly higher than that of blank cells ($p < 0.05$), only for damaged and decontaminated skin, reaching values below 4.5 ng/cm^2/24h. In decontaminated skins, Co permeation started at 1 h, while for broken skin, the permeation started after 8 h of exposure. Broken skin allowed skin permeation of Cr to reach values similar to those of Ni, whereas in decontaminated cells, an amount of approximately 50 ng/cm^2/24h was found. Cu skin penetration reached 200 ng/cm^2/24h in decontaminated cells and a higher level in broken skin. Mo skin permeation was negligible for intact skin, but increased significantly for decontaminated, damaged, and broken skins ($p < 0.05$) reaching 47.8 ± 42 ng/cm^2/24h. For Mn, which presented the highest dissolution (Table 1), permeation reached the highest value for broken skin (3148 ± 2650 ng/cm^2/24h, followed by decontaminated skin at 8 h, decreasing to 578 ± 509 ng/cm^2 at 24 h ($p < 0.05$). It should be noted that for all metals except Mn and Mo, cells decontaminated using soaps and water showed the higher permeation profile and, for broken skins, the increment of permeation occurred after 8 h linearly for all metals except for Mn, for which the increase started immediately.

The metal contents inside the skin are shown in Fig. 2 and Table 2. The metal most represented inside the skin was Cr, with more than 15 μg/cm^2/24h for intact skin (epidermis) and more than 20 μg/cm^2/24h for damaged and broken skin (higher for dermis, $p < 0.05$). Ni inside the skin reached the 10.2 ± 8.5 μg/cm^2/24h for intact skin ($p < 0.05$ with blank) and were over 14 μg/cm^2/24h for damaged and broken skins. Lower skin content was found for Mo, Mn, Cu and Co with a concentration in intact skin of 2.0 ± 1.7, 1.7 ± 1.3, 0.7 ± 0.5 and 0.6 ± 0.1 μg/cm^2/24h respectively.

As expected, the metal content was lower in decontaminated skins because the donor solution was removed after a short exposure time.

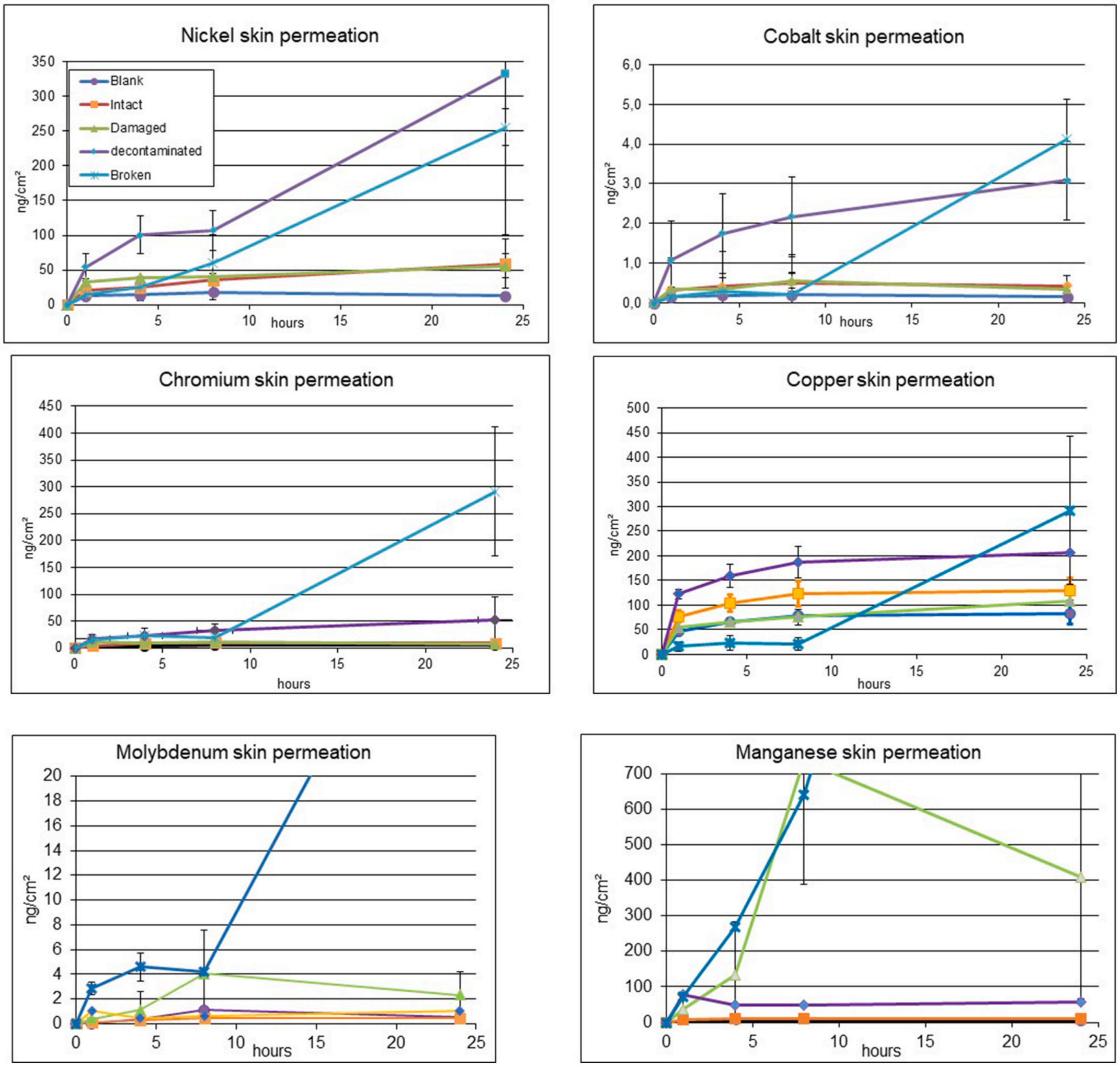

Fig. 1. Metals skin permeation profiles detected in receiving phases.

4. Discussion

This study investigated the skin penetration and permeation of metals released from hydrogenated stainless steel particles derived from the dismantling procedures of nuclear power plants. The aim was to determine the skin absorption of the metals contained in these particles. This study was preliminary to the study of tritiated particle skin absorption.

The effects of metals released from stainless steel particles have been investigated in marine bivalve molluscs (Vernon et al., 2022) and lung cells (Lamartiniere et al., 2022), but no data are available on skin penetration and permeation.

The first result of our study was the higher permeation profile for broken skin (as expected), but also for decontaminated skin. In the latter case, we have a reduction in metal skin content, while the treatment causes an increase in metals found in the receiving phase. The increase of permeation profile in skin treated with soaps to remove the contaminant was already demonstrated in a previous study on lead permeation (Filon et al., 2006) in which a median penetration of 2.9 ng/cm^2/24h was found in non-treated skin *vs* 23.6 ng/cm^2/24h in decontaminated skin. This effect was common for all metals tested (except Mo) and can be explained by the surfactant action of soaps on stratum corneum integrity (Franklid et al. 1995, Lindberg et al., 1989; Emilson et al., 1993; Magnano et al., 2021) to enhance skin absorption of chemicals (Filon et al., 2006; Sun et al., 2002; Ahlstrom et al., 2018). This result highlights the need to protect the skin to avoid contamination and use only water to decontaminate the skin after contact with metals (Magnano et al., 2021).

As expected, broken skin caused an increase in metal permeation, although this effect started after 8 h of exposure to Ni, Cr, Co, and Cu, probably due to an elastic effect of the skin, for which only after some hours the hole became effective for metal passage. To the best of our knowledge, no data are available for this method.

In our study, Ni, Co, and Cr permeation was lower compared to previous data (Magnano et al., 2023, 2024; Filon et al., 2009) because of the lower amount of metals in the donor phases. For Cu, Mn and Mo, to the best of our knowledge, no comparative data are available.

Considering the toxicological profiles of metals released from the stainless steel particles, the dose permeated through exposed skin (hands, neck, and face: approximately 40 cm^2) for 8 h was very low for intact skin, but more relevant in the case of incorrect decontamination with water and soap or broken skin. Data collected from workplaces demonstrate that metals may enter systemic circulation through the dermal route (Scansetti et al., 1994; Sun et al., 2002; Klasson et al.,

Table 2
Metals in receiving solution and into the skin after 24 h (mean ± standard deviation).

Time (h)	Ni	Co	Cr	Cu	Mn	Mo
Receiving solution ng/cm^2 at 24 h						
Blank	13.1 ± 4.9	0.2 ± 0.2	5.1 ± 0.3	83.6 ± 21.4	5.6 ± 1.9	1.3 ± 0.6
Intact	59.2 ± 35.2*	0.4 ± 0.2	8.5 ± 4.2	130 ± 24.5*	9.6 ± 2.3*	1.3 ± 0.1
Damaged	56.2 ± 17*,ˆ	0.4 ± 0.3	7.0 ± 4.0	107.6 ± 2.4*	408 ± 343*,ˆ	2.9 ± 2.3*,ˆ
Decontaminated	255.3 ± 26.7*,^{1}	3.1 ± 2.3*,ˆ	51.4 ± 43.7*,ˆ	206 ± 11.1*,ˆ	578 ± 509*,ˆ	2.2 ± 0.3*,ˆ
Broken	332 ± 32*,ˆ	4.1 ± 2.6*,ˆ	290 ± 120*,ˆ	291 ± 150*,ˆ	3148 ± 2650*,^{1}	47.8 ± 42*,ˆ
Skin content µg/cm^2						
Blank	**Ni**	**Co**	**Cr**	**Cu**	**Mn**	**Mo**
Epidermis	0.06 ± 0.02	0.001 ± 0.002	0.2 ± 0.03	0.05 ± 0.01	0.02 ± 0.003	0.001 ± 0.002
Dermis	0.2 ± 0.1	0.004 ± 0.005	3.1 ± 3.8	0.20 ± 0.01	0.2 ± 0.2	0.004 ± 0.005
Total	0.3 ± 0.1	0.005 ± 0.007	3.2 ± 3.8	0.3 ± 0.002	0.2 ± 0.2	0.005 ± 0.007
Intact						
Epidermis	9.0 ± 7.7*	1.8 ± 1.5*	14.2 ± 12.3*	0.5 ± 0.4*	1.4 ± 1.2*	1.8 ± 1.5*
Dermis	1.3 ± 0.7*	0.2 ± 0.2	2.3 ± 1.6	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.2
Total	10.3 ± 8.5*	2.0 ± 1.7*	16.5 ± 13.9*	0.7 ± 0.5*	1.7 ± 1.3*	2.0 ± 1.7*
Damaged						
Total	15.1 ± 4.3*	3.0 ± 0.9*	23.6 ± 12.0*	1.0 ± 0.02*	5.8 ± 1.1	3.0 ± 0.9*
Decontaminated						
Epidermis	1.4 ± 1.5*	0.3 ± 0.3	2.1 ± 2.5	0.1 ± 0.1	0.2 ± 0.2	0.3 ± 0.9*
Dermis	2.0 ± 1.0*	0.4 ± 0.2*	3.1 ± 1.6	0.3 ± 0.1	0.3 ± 0.2	0.4 ± 0.4*
Total	3.3 ± 0.5*	0.7 ± 0.5*	5.3 ± 2.9	0.4 ± 0.2*	0.5 ± 0.4	0.7 ± 0.1*
Broken						
Epidermis	4.9 ± 3.5*	1.0 ± 0.4*	7.7 ± 4.0*	0.3 ± 0.2	2.7 ± 1.3*	1.0 ± 0.4*
Dermis	9.9 ± 6.5*	1.9 ± 1.2*	15.3 ± 12.0*	0.8 ± 0.3*	5.1 ± 3.3*	1.9 ± 1.0*
Total	14.8 ± 10.5*	2.9 ± 2.0*	23.0 ± 12.0*	1.1 ± 0.5*	7.8 ± 4.3*	2.9 ± 1.9*

Mann-Whitney : *$p<0.03$ between sample and blank ˆ$p<0.03$ between sample and intact skin

2017; Kettelarij et al., 2018), although the level is generally low, and the local effect on the skin could be more relevant, mainly for sensitizing metals. The Food and Drug Administration defined the level of metals in cosmetics for which an adverse effect is not expected (Hepp et al., 2014) that are higher than the values obtained in the present study (3.1 mg/kg, Cr, 0.9 mg/kg of Co, and 2.7 mg/kg of Ni). From this point of view, contact of the skin with stainless steel particles cannot cause adverse effects on intact skin.

However, the presence of Ni, Cr, and Co can cause contact sensitization with the induction of allergic reactions and possible onset of allergic contact dermatitis in subjects already sensitized to these metals. No effect is known for Cu, Mn, and Mo, for which the sensitization potential can be considered low.

As expected, the concentration of metals inside the skin was higher for broken and damaged skin and lower for decontaminated skin. Impairment of the stratum corneum causes an increase in metal skin absorption as well as an increase in skin diseases such as allergic contact dermatitis. The metal content was higher in the epidermis of intact skin and higher in the dermis of broken skin.

Moreover, it is possible a significant retention of the metals inside the skin (i.e. in hair follicles, sweat glands), possibly forming reservoirs where internal exposure may continue for extended periods of time, even after external exposure has ended. The binding of metals to skin components can decreases permeability. To note that the permeation of metals is most likely influenced by the pH of the donor solution and by the valence state of the metals. Another important point to be considered is the presence of counter ions such as chloride that could influence permeability of metals (Franken et al., 2015)., In terms of metal concentrations inside the skin, our results are in line with those of previous studies on the skin absorption of Ni, Co, and Cr (Magnano et al., 2023; Magnano et al., 2024; Filon et al., 2009). Moreover, Hagvall et al. (2021) found that Ni ions penetrate the stratum corneum and can reach the upper parts of the epidermis, causing allergic sensitization. This author confirmed that also Cr and Co, applied on the skin as salts, accumulated mainly in the stratum corneum, but could also be detected in epidermis. Co and Cr(III) species penetrated into the epidermis to a larger extent than nickel species.

Considering the levels of metals that penetrate the skin, a short contact with the skin can cause the deposition of Ni from nickel-containing materials (Erfani et al., 2015) with potential allergic reactions in nickel-sensitized as well as after three repeated exposure for 10-min to solid nickel discs (Ahlstrom et al., 2018). Regarding levels of Ni that can cause allergic reaction, the EU Directive on Ni (94/27/CE and 2004/96/CE) fixed the value of Ni release of 0.2 μg/cm^2/week, for piercing and 0.5 μg/cm^2/week for other products that can come into direct and prolonged contact with the skin. These values can prevent the induction of allergic contact sensitization.

In our study, all cells treated with stainless steel particles presented levels over this value after 24 h, suggesting the potential for skin sensitization in the case of short (decontaminated cells) and long-term contamination. with Ni dose above the 0.5 μg/cm^2. Ahlstrom et al. studied Ni deposition and penetration after short contact with a metal object containing Ni in subjects sensitized and non-sensitized to this metal (Ahlstom, 2019). Inside the stratum corneum, they found Ni levels of 4.1 μg/cm^2 after 3 min of contact and 0.6 μg/cm^2 after 24 h in irritated skin with soap and 7.7 μg/cm^2 after 3 min of contact and 1.1 μg/cm^2 in normal skin. Among the 16 exposed workers sensitized to Ni, these concentrations were sufficient to trigger allergic contact dermatitis in 10 treated and 3 untreated workers with soap. No reaction was observed in the non-sensitized subjects (Ahlstrom et al., 2019). Moreover, (Erfani et al., 2015) Erfani et al. demonstrated in 2005 that Ni can be found on skin surface after a single touch of SS 316 L as well as of other Ni containing alloys with a dose ranging between 0.024 and 4.7 μg/cm ^{2}.

Ni levels found in the donor solution and in intact, damaged, and broken epidermis are similar to the results of Ahlstrom et al. (2019), suggesting the potential for SSP to induce contact dermatitis in already sensitized patients Therefore, it is important to protect the skin to avoid contact with the powder. To be note, the use of a protective cream prior to potential exposure can limit metal absorption through the skin (Magnano et al., 2023, 2024).

The minimum elicitation threshold concentration for Cr suggested is 0.2 μg/cm^2/48h (Hansen et al., 2003, 2006; Hedberg et al., 2010) that was reached in all experiments performed after 24 h of exposure.

Regarding Co, Summer et al. (2007), did not find patch-test reactions to stainless steel in 16 cobalt-sensitized patients. However, (Julander et al., 2009) reported that 1.2 mg/cm^2 of Co released on the skin can elicited allergic response in cobalt-sensitized patients. In our study, Co exceeded this value in all skin samples except those decontaminated.

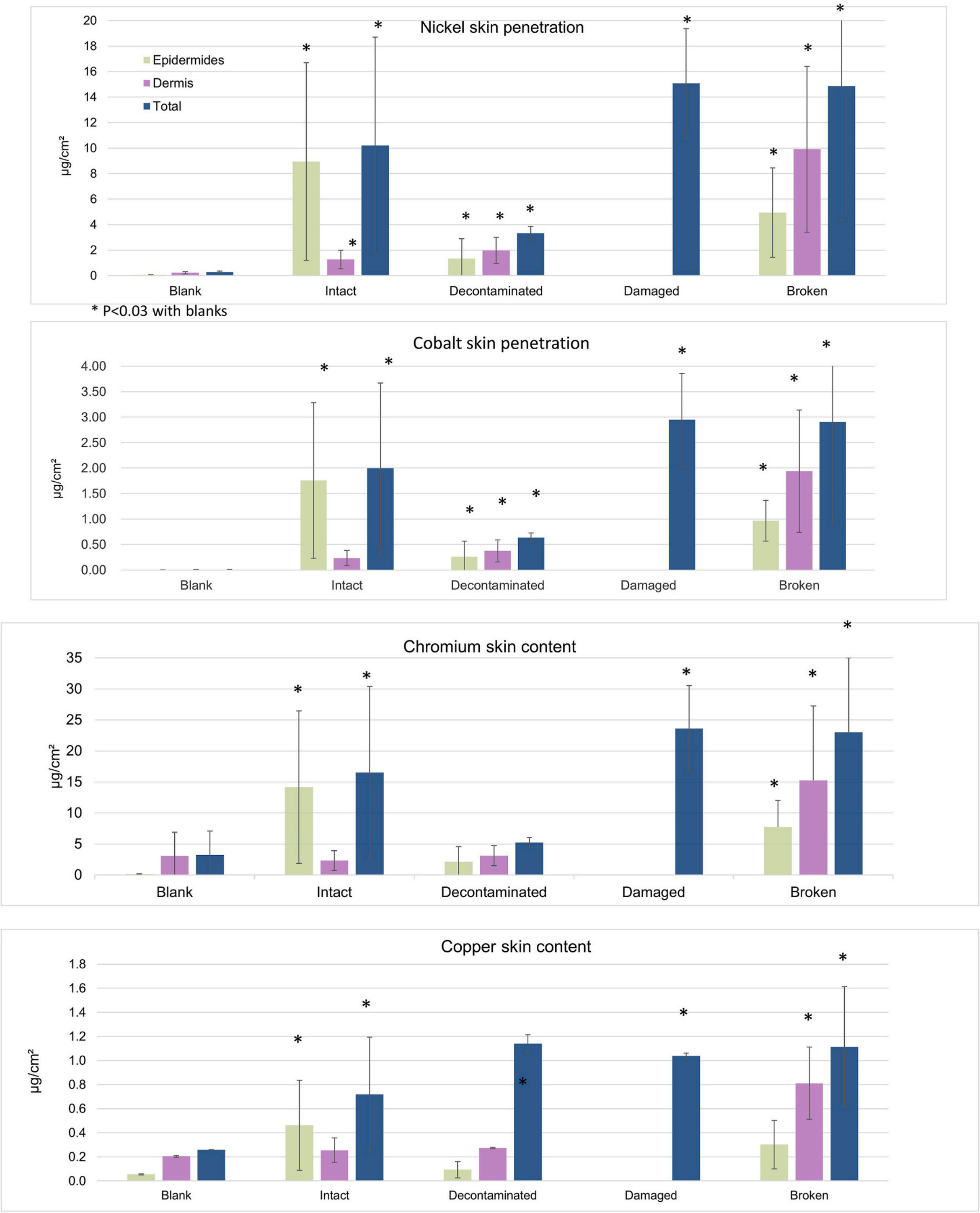

Fig. 2. Metal content into the skin in experiments performed.

The next step of our study is to assess tritium penetration and absorption after exposure to tritiated particles. Both sets of data will be useful for defining hazards related to exposure to tritiated stainless steel particles.

4.1. Limitation of the study

The *in vitro* evaluation of metal penetration and permeation can be affected by high variability due to donor skin characteristics, storage procedures, and treatments. Moreover, high hydration of the skin for 24 h can cause an increase in skin permeation, thereby overestimating skin penetration and permeation. The protocols used to damage or break the skin can be affected by manual differences between operators, although we tried to standardize the method and the same operators performed all the skin lesions, according to the procedures applied in long experience on these experiments (Filon et al., 2009). Moreover, in the analysis of Cr we did not considered speciation, but data in literature reports the release mainly of Cr(III) from SS 316L (Taxell et al. 2022).

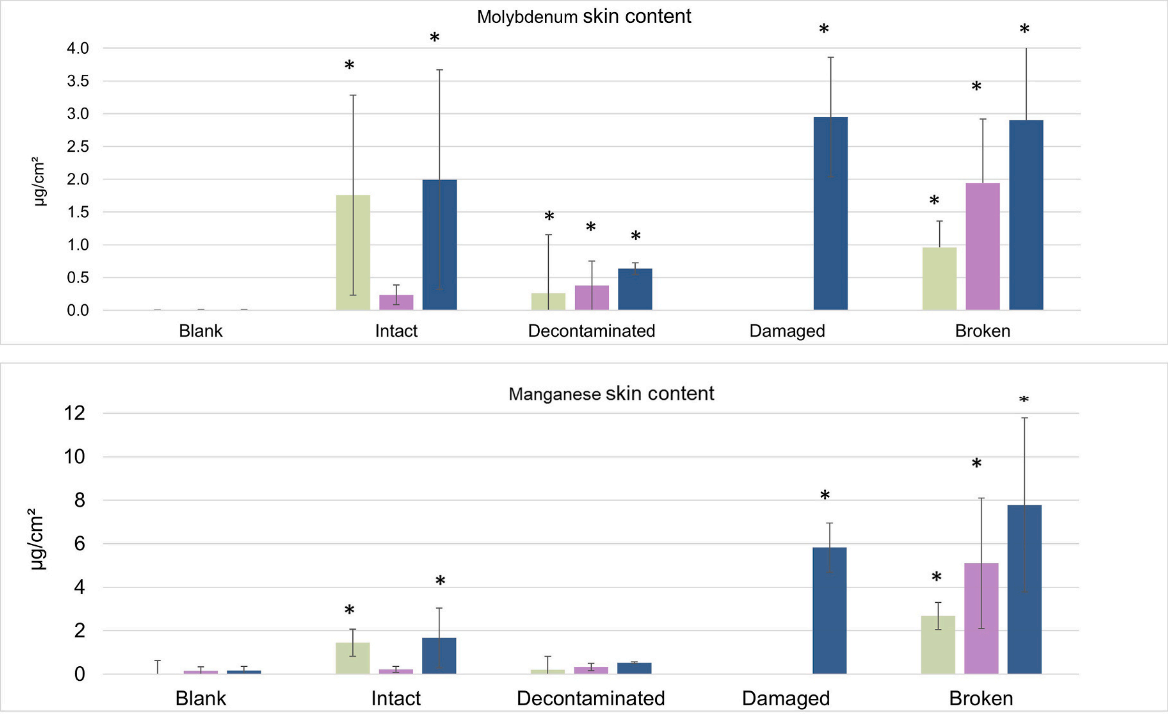

Fig. 2. (*continued*).

5. Conclusions

Our study evaluated the release of metals and their skin penetration and permeation using hydrogenated stainless-steel particles produced during the dismantling of nuclear power plants.

The main finding was an increase in metal permeation after a short contact with powders and a decontamination procedure with soap, suggesting the need to avoid contact with powders and to use only water in case of contact. Overall, the levels of metals that reached the receiving solution were very low in the case of intact (and damaged) skin contact, and significantly increased only in the case of broken and decontaminated skin.

The skin content of sensitizing metals (Ni, Cr, and Co) could induce allergic sensitization or cause allergic contact dermatitis in subjects already sensitized to Ni. Because of the wide literature on this topic, the levels found for this metal are comparable with those of other studies on the sensitization potential of Ni concentrations (Ahlstrom et al., 2018, 2019).

Preventing skin contact with these powders is suggested using protective measures such as Tyvek coveralls with long sleeves, gloves, goggles, and respirators for exposed workers. For accidental exposure, adverse effects are not likely, except for subjects already sensitized to Ni, for which allergic contact dermatitis could develop in the exposed skin.

CRediT authorship contribution statement

Francesca Larese Filon: Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Giovanna Marussi:** Writing – review & editing, Visualization, Resources, Investigation. **Mickael Payet:** Writing – review & editing, Formal analysis. **Olivier Debellemaniere:** Writing – review & editing, Formal analysis. **Pier Camillo Parodi:** Writing – review & editing, Formal analysis. **Nicola Zingaretti:** Writing – review & editing, Formal analysis. **Veronique Malard:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Laurence Lebaron-Jacobs:** Writing – review & editing, Supervision. **Gianpiero Adami:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Marcella Mauro:** Writing – review & editing, Formal analysis, Data curation. **Elena Pavoni:** Writing – review & editing, Formal analysis. **Matteo Crosera:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Francesca Larese Filon reports financial support was provided by EU Framework Programme for Research and Innovation Euratom. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union. Project TITANS EURATOM https://titans-project.eu/the-project/.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.125327.

Data availability

Data will be made available on request.

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