



Titre: Comparison of Synthetic Sweat and Influence of Sebum in the Permeation of Bioaccessible Metal(loid)s from Contaminated Soils through a Synthetic Skin Membrane
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1 **Comparison of synthetic sweat and influence of sebum in the permeation of**
2 **bioaccessible metal(loid)s from contaminated soils through a synthetic skin membrane**

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8

9 **Abstract**

10 Dermal exposure to metal(loid)s from contaminated soils has received less attention than oral
11 and inhalation exposure. Still, it can be a relevant pathway for some contaminants.
12 Comparison of synthetic sweats (donor solutions), the influence of sebum, and the
13 characterization of diffusion parameters through a synthetic membrane (acting as skin
14 surrogate) in the permeation of metal(loid)s (As, Cr, Cu, Ni, Pb, and, Zn) from polluted soils
15 is missing. Dermal bioaccessibility test were performed using two sweat compositions (EN
16 1811, pH 6.5 (Sweat A) and NIHS 96-10, pH 4.7 (Sweat B)). Diffusion parameters of soluble
17 metal(loid)s using the Franz cell methodology were calculated using the Strat-M membrane.
18 The influence of synthetic sebum in the permeation of metal(loid)s was also investigated.
19 The metal(loid) bioaccessibility percentage was higher for Sweat B (pH 4.7) compared to
20 Sweat A (pH 6.5), attributed to lower pH of sweat B. Among the six elements tested, only
21 chromium and copper permeated the membrane. Permeation coefficient (K_p) was higher for
22 chromium in Sweat A (0.05 to 0.11 cm h^{-1}) than Sweat B (0.0007 to 0.0037 cm h^{-1}) likely
23 due to a higher pH and thus more permeable Cr species. Presence of sebum increased lag
24 times for copper permeation. Additional studies regarding speciation of metal(loid)s

25 following extractions in synthetic sweat and comparison of synthetic membrane Strat-M and
26 human skin in the permeation of metal(loid)s are recommended.

27

28 **Key words:** contaminated soils, dermal permeation, Franz diffusion cell, in vitro dermal
29 bioaccessibility, synthetic sebum, Strat-M membrane, synthetic sweat.

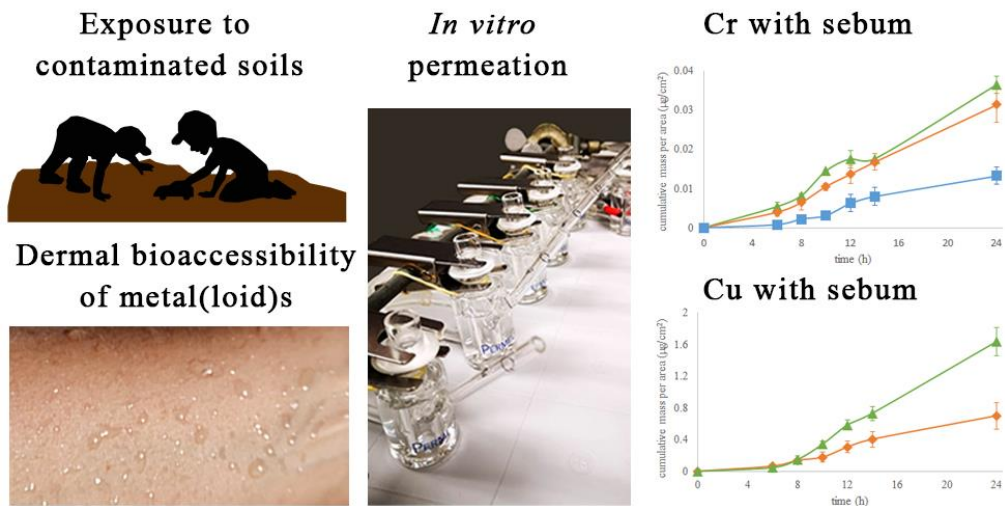
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31 **Synopsis:** When metal(loid)s found in polluted soils are extractable in sweat they don't
32 indeed permeate a synthetic skin membrane

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34 **Graphical abstract TOC**

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42 **1. Introduction**

43 Humans can be exposed to toxic metal(loid)s in various environmental media, including
44 soils, via the dermal pathway. The majority of scientific studies have focused on oral
45 bioaccessibility of pollutants (ingestion pathway) and more recently, on *in vitro* test
46 development and application for assessing inhalation bioavailability ¹⁻⁴ and *in vitro* dermal
47 bioavailability. ⁵⁻⁸

48 It is generally acknowledged that there are very few quantitative and qualitative data on
49 dermal exposure to toxic chemicals present in geological materials ⁹. Additionally, some of
50 the permeation data of chemicals through the skin were obtained using different experimental
51 conditions in terms of concentration in the donor solution, type of membrane (animal, ex
52 vivo), and duration, making a comparison of results difficult. ¹⁰ Quite the reverse, there is a
53 copious amount of data regarding the passage of drugs and cosmetics through the skin.
54 However, very little is known about this route of entry for environmental contaminants bound
55 to geological materials. The technology used to study the percutaneous penetration of drugs
56 could be used to assess dermal penetration of toxic compounds as well. ⁹⁻¹¹

57 The most common method to evaluate dermal diffusion was introduced by Franz ¹² in 1975.
58 This method has been adapted and modified by many researchers to study the dermal
59 pathway for drugs, cosmetics, and chemicals due to its low cost, short time, and good
60 reproducibility. ⁹

61 Franken et al.¹⁰ highlighted the importance of the donor solution composition in the Franz
62 cell methodology. The donor solution should be standardized since certain components may
63 promote or inhibit oxidation of metal(loid)s. There is no standardized donor solution for
64 permeation studies, and to date, a comparison of the influence of the synthetic sweat

65 composition as donor solution in the permeation of metal(loid)s from geological materials is
66 missing. Recent studies reported that synthetic sweat composition strongly influences the
67 solubility of metals from geological materials ⁷⁻⁸. Even though the influence of synthetic
68 sweat formulation in the solubility of metals has been demonstrated, the impact of synthetic
69 sweat characteristics when used as donor solution in the dermal permeation of metal(loid)s
70 from geological materials is not clear.

71 Different membranes to simulate human skin have been used for dermal permeation studies
72 using the Franz cell methodology. According to the OECD and USEPA guidelines, human
73 skin is the "gold standard" ⁶. Unfortunately, human skin is not always available, can be
74 expensive, and is highly variable depending on age, sex, origin of the donor, and body area.¹³
75 Because of these limitations, some studies used animal skin, such as swine, rat, or guinea pig,
76 as human skin surrogates. ^{10,14} Animal skin as a surrogate for human skin also has limitations
77 such as high variability inherent to biological membranes (coefficient of variation = 72%)
78 rendering the experimental design, and ability to compare results difficult. ^{13,15} Moreover,
79 ethical consideration inherent to the use of biological tissues from animal origin and in vivo
80 experiments with animals must be considered.

81 Artificial membranes used to model skin permeation should mimic the stratum corneum as
82 close as possible, have low variability and be commercially available. ¹⁶ The Strat-M (EMD
83 Millipore, MA, USA) synthetic membrane is an ultrafiltration membrane made of
84 polyethersulfone composed by multiple layers, including two layers impregnated with
85 synthetic lipids and a very tight top layer, producing diffusion results similar to natural human
86 skin. ¹⁶⁻¹⁷ In diffusion studies with human cadaver skin, animal skin, and Strat-M. membrane,
87 Joshi et al.¹⁷ tested the Strat-M membrane with a mixture of synthetic lipids (to mimic the

88 lipid phase in human skin) in the permeation of nicotine and hydrocortisone. The authors
89 reported that when the membrane Strat-M was treated with synthetic lipids, it showed a closer
90 correlation to human skin than the untreated membrane and animal skin. They also exposed
91 the difficulties of correlating diffusion data between animal skin and human skin ¹⁷. In most
92 cases, the correlation between the treated synthetic membrane Strat-M and human skin was
93 better. Moreover, Strat-M membrane showed a high lot-to-lot reproducibility and high shelf
94 life as opposed to human and animal skin. Yet, to our knowledge, the use of the membrane
95 Strat-M to assess the dermal permeation of metal(loid)s from contaminated geological
96 materials has not been reported yet.

97 The dermal bioaccessible fraction of a chemical is the amount that is dissolved in sweat and
98 is available for penetration through the skin.^{5,18} This concentration can be used in conjunction
99 with the permeation data to calculate bioavailability. ⁵

100 To estimate the dermal absorption of contaminants from aqueous media, the USEPA ¹⁹
101 proposes the water approach methodology. It assumes contact with contaminated water and
102 aims to calculate the dermally absorbed dose (DAD) using the migration rate of a chemical
103 through the skin. This migration is characterized by the permeability coefficient K_p (cm h^{-1}).
104 This coefficient is available in the literature for several inorganics and originates from
105 experimentally measured or derived values. ^{7,19} Nevertheless, published K_p values involve a
106 high level of uncertainty (since they don't take speciation into account except for chromium)
107 and they are available for metal(loid)s soluble in water but not soluble in sweat. ¹⁹
108 Improvement in K_p determination can reduce uncertainty in the calculation of dermal
109 exposure therefore refining exposure assessment. To our knowledge, the influence of

110 synthetic sweat formulation on K_p evaluation to assess the dermal permeation of metal(loid)s
111 from contaminated soils has not been reported to date.

112 Therefore, the present study aims to (1) assess and compare the dermal bioaccessibility of
113 As, Cr, Cu, Ni, Pb, and Zn present in various geological materials via *in vitro* experiments
114 using two artificial sweat formulations; (2) evaluate the diffusion parameters of the
115 bioaccessible fraction of these metal(loid)s through artificial membrane Strat-M using the
116 static Franz cell methodology; and (3) investigate the influence of synthetic sebum in the
117 permeation of these metal(loid)s through artificial membrane Strat-M.

118 **2. Materials & Methods**

119 **2.1 Soil sampling and characterization**

120 Three geological materials have been subjected to *in vitro* bioaccessibility tests to assess the
121 dermal bioaccessible fraction of As, Cr, Cu, Ni, Pb, and Zn. The certified material SQC001
122 (lot number LRAC0025, produced by Sigma-Aldrich in accordance with ISO 17034
123 and ISO/IEC 17025 procedures ($d < 425 \mu\text{m}$)), and two field-collected soil samples (S7 and
124 S8), sampled near Chromated copper arsenate (CCA)-treated utility poles in the Montreal
125 area (Quebec, Canada). The soils were sampled in a 20 cm radius of the poles and up to 10
126 cm depth. Coarse material ($>2 \text{ cm}$) and topsoil vegetation were removed prior to sampling.
127 The samples were collected using a plastic shovel and stored in zip-lock plastic bags.
128 Containers and tools were washed with a phosphate-free detergent and soaked overnight in
129 10% ($v v^{-1}$) HNO_3 and rinsed with deionized water ($18.2 \text{ M}\Omega\cdot\text{cm}$) prior to use.²⁻³ Field-
130 collected soil samples were air-dried, gently disaggregated using a mortar and dry sieved to
131 $420 \mu\text{m}$ using a sieve shaker (Retsch AS-200). Samples were then stored at 4°C .

132 Total metal(loid) content in soil samples was determined via acid digestion on a hot plate
133 using HNO₃ (70 % w/w), HF (50 % w/w), and HClO₄ (70 % w/w) according to standard
134 method 3030.²⁰ Digestates were transferred to 100-ml volumetric flasks and made up to
135 volume with deionized water. Solutions were filtered (0.45µm) with glass microfiber filters
136 (Whatman) and stored in polypropylene centrifuge tubes with HDPE screw caps. Cr, Cu, Ni,
137 Pb, and Zn concentrations were measured via atomic absorption spectroscopy (AAS)
138 (Perkin- Elmer A200). Detection limits (DLs, determined based on signal-to-noise approach
139 (ratio of 3:1)) in mg kg⁻¹ were 0.3, 0.3, 0.2, 1, and 0.1, respectively. Arsenic content was
140 determined via ICP-OES (Varian Vista), with a detection limit (DL) of 0.004 mg kg⁻¹. Soil
141 pH was measured in duplicates in solid-to-liquid ratio 1:2 with deionized water (pH meter:
142 Eutech pH 200 series, probe: Accumet Ag/AgCl) according to method ASTM D4972-13.²¹
143 Total organic carbon content with a detection limit (DL) of 0.1% w/w was analyzed using a
144 LECO furnace. Infrared determination of CO₂ was achieved to determine organic carbon
145 content as a difference between total and inorganic carbon.²² Cation exchange capacity
146 (CEC) was determined using the sodium acetate method with NaOAc 1N and NH₄OAc 1N.²³

147 **2.2 Artificial SSFLs**

148 Three solutions have been prepared to mimic human skin surface film liquids (SSFL): two
149 synthetic sweats (Sweat A (pH = 6.5) and Sweat B (pH = 4.7)) and one synthetic sebum
150 (Table 1). The SSFL formulations are further described elsewhere⁸ and have been selected
151 for their differences in pH and composition. Sweats A and B simulate the sweat layer on the
152 skin, while sebum was used to treat the synthetic membranes to mimic the hydrophobic
153 properties of the skin, caused by the presence of lipids.

154 **Table 1**

155 Composition of artificial SSFLs

Chemical, % (w/w)	Sweat A ^a (pH = 6.5)	Sweat B ^b (pH = 4.7)	Sebum ^c
Deionized water	99.3	94	-
Sodium chloride	0.5	2	-
Lactic acid	0.1	1.5	-
Urea	0.1	0.5	-
Acetic acid	-	0.25	-
Ammonium chloride	-	1.75	-
Squalene	-	-	12.4
Jojoba oil	-	-	25
Triolein	-	-	44.6
Oleic acid	-	-	17
Vitamin E	-	-	1

156

157 ^a According to standard EN 1811 ²⁴158 ^b According to standard NIHS 96-10 ²⁵159 ^c According to Wertz ²⁶

160

161 **2.3 *In vitro* dermal bioaccessibility test**

162 Tests were started by adding 20 ml of synthetic sweat to 2 g of soil sample ($d < 425 \mu\text{m}$) in
163 50 ml Polypropylene tubes with HDPE caps. Tubes containing the soil and sweat mixture
164 were placed on an orbital shaker (Cole-Parmer 51704 Series, radius 9.5 mm) at 100 rpm
165 inside an incubator (Isotemp, Fisher Scientific) at 36°C, corresponding to the median skin
166 temperature for humans ⁵, for 2 hours. The tubes were then centrifuged (Heraeus Megafuge
167 8, Thermo Fisher) at 10,000 x g for 10 minutes, and the supernatant collected with 60 ml
168 Luer-Lok syringes and filtered with a 0.45 μm PVDF filter fitted to the syringe. The filtered
169 supernatant was transferred into 50-ml Polypropylene centrifuge tubes with HDPE caps and
170 stored at 4°C until analysis. Samples were analyzed via ICP-AES (Vista, Varian Inc.) to
171 determine As, Cr, Cu, Ni, Pb, and Zn concentrations (with detection limits in mg kg^{-1} of

172 0.004, 0.001, 0.006, 0.001, 0.006, and 0.024, respectively). For each metal(loid),
173 bioaccessibility percentage (%_{bio}) was determined as follows:

$$174 \quad \%_{bio} = \frac{C_{bio}}{C_{total}} * 100 \quad (1)$$

175 Where C_{bio} is the bioaccessible concentration of metal(loid) (mg kg⁻¹), and C_{total} is the total
176 concentration of metal(loid) in the soil sample (mg kg⁻¹). A more detailed description of the
177 dermal bioaccessibility protocol can be found elsewhere.⁸

178 **2.4 Permeation test**

179 Glass jacketed vertical Franz diffusion cells (PermeGear Inc.) with a 9 mm orifice diameter,
180 5 ml receptor volume, and 1 ml donor volume were used. The temperature of the receptor
181 was maintained at 37 °C by circulating water from a water bath (Model 2849, Thermo Fisher
182 Scientific) to simulate temperature below the skin.^{10, 27} The receptor compartment was filled
183 with Phosphate-Buffered Saline (PBS) solution (Fisher Scientific) at a pH of 7.4¹³ and NaCl
184 8.0 g/L, KCl 0.2 g/L, Na₂HPO₄ 1.44 g/L, and KH₂PO₄ 0.24 g/L, to represent blood salt
185 concentration and blood pH.¹⁰ The receptor compartment was subjected to constant stirring
186 (300 rpm) with a magnetic stirrer (Poly 15, Variomag). Twenty-five mm OD sterile Strat-M
187 membranes (EMD Millipore) were used as a surrogate for human skin. Each membrane was
188 mounted on the Franz diffusion cell with the shiny side in contact with the donor
189 compartment.¹³ To simulate the hydrophobic character of the skin and investigate the
190 influence of the lipid fraction of SSFL in the permeation of metal(loid)s, half of the
191 membranes were coated with 0.1 ml of sebum.

192 One ml of filtered supernatant collected from the bioaccessibility test (donor solution) was
193 immediately added to the open-top donor compartment to start the permeation experiment.

194 Tests were performed in duplicate and in the presence of procedure blanks (fresh synthetic
195 sweat as donor solution). The receptor solution was completely removed from the receptor
196 compartment at 6, 8, 10, 12, 14, and 24 h and placed in 15 ml Polypropylene centrifuge tubes.
197 After each sampling event, the receptor compartment was rinsed with 5 ml of fresh PBS
198 solution using a syringe. This rinsing solution was added to the tube containing the receptor
199 solution. After every sampling event followed by rinsing, 5 ml of fresh PBS solution was
200 added to the receptor compartment. The procedure blank cells received the same treatment.
201 The receptor and donor solution samples were kept refrigerated at 4°C until analyzed via
202 ICP-AES (Vista, Varian Inc.) to determine As, Cr, Cu, Ni, Pb and Zn concentrations.
203 As an additional quality control measure, a mass balance was performed for one sample per
204 batch. At the end of the permeation test (after 24 h), the donor compartment and the
205 membrane were rinsed four times with 1 ml of deionized water. The rinsing solution was
206 added to the remaining donor solution for analysis. To assess the amount of metal that
207 remained in the membrane, membranes were placed in Teflon beakers and digested on a hot
208 plate with HNO₃, HCl, and HF for 45 minutes and then filtered with 0.45 µm filters
209 (Whatman), diluted to 100 ml and analyzed for total metal content via ICP-AES. Mass
210 balance was calculated by comparing the metal recovered from the donor solution,
211 membrane, and receptor solution at the end of the experiment with the initial mass of metal
212 present in the donor solution.

213 **3. Results and Discussion**

214 **3.1 Soil Characterization**

215 The pH was neutral to slightly alkaline for CCA-contaminated soil samples S7 and S8 and
216 acidic for reference material SQC001 (Table 2). Low total organic carbon content (<2.9%)

217 was reported in all soil samples. CEC values ranged from 12.8 to 41.4 meq 100 g⁻¹. High
 218 CEC suggests that cationic metals such as Cu, Ni, and Zn could be retained by cation
 219 exchange on the soil. ² Total metal(loid)s concentrations in soil samples S7 and S8 are also
 220 shown in Table 2. Values in bold are exceeding Quebec's regulatory limit for industrial land-
 221 use (C criterion).²⁸ Extensive As contamination was observed for S8 (1639 mg kg⁻¹ ± 6.8 %) (more than 30 times the C criterion) and S7 (311 mg kg⁻¹ ± 1.1 %). Cu content exceeding the
 223 C criterion (500 mg kg⁻¹) was also observed for S7 (824 mg kg⁻¹ ± 5.8 %) and S8 (1070 mg
 224 kg⁻¹ ± 11.0 %). As previously reported, Cr contamination was less problematic than As and
 225 Cu ² but soil samples S7 and S8 still contained elevated Cr concentrations. Certified reference
 226 material SQC001 had a lower content of As, Cr, and Cu but a higher content of Pb, and Zn.
 227 The measured total metal(loid) content of SQC001 was within 100 ± 10% of the certified
 228 values provided in the certificate of analysis.

229

230 **Table 2**

231 Total concentrations of As, Cr, Cu, Ni, Pb, and Zn (mg kg⁻¹), pH, total organic carbon (TOC,
 232 w/w %), and cation exchange capacity (CEC, meq 100 g⁻¹) of soils. Precision is expressed
 233 as mean ± relative standard deviation %.

Parameter	SQC 001	S7	S8
As	173 ± 20	311 ± 1.1	1639 ± 6.8
Cr	124 ± 6.5	371 ± 3.9	582 ± 14.3
Cu	82 ± 1.9	824 ± 5.8	1070 ± 11.0
Ni	112 ± 9.8	26 ± 11.5	223 ± 11.3
Pb	263 ± 2.5	57 ± 8.8	80 ± 10.6
Zn	512 ± 4.0	261 ± 7.3	223 ± 10.8
pH	5.7 ± 0.2	7.2 ± 0.7	7.1 ± 0.5
TOC	< 0.01	1.3 ± 2.1	2.9 ± 1.5
CEC	12.8 ± 8.8	15.5 ± 16.3	41.4 ± 2.8

234

235

236 **3.2 Dermal bioaccessibility of metal(loid)s**

237 Table 3 shows the bioaccessibility (%) and bioaccessible concentration (mg l^{-1}) of
238 metal(loid)s obtained following bioaccessibility tests performed on S7, S8 and SQC001 using
239 Sweat A and Sweat B. In agreement with the findings of Marin Villegas et al. ⁸, Sweat B
240 generally yielded the highest bioaccessibility percentage values. The difference in the
241 bioaccessibility of metal(loid)s for the different sweat compositions can be attributed to the
242 fact that lower pH increases the solubility, particularly for cationic metals.

243 The reference material SQC001 revealed an overall higher dermal bioaccessibility
244 percentage compared to S7 and S8, especially for Cu, Ni, Pb, and Zn. In sweat B, which
245 yielded higher bioaccessibility percentages than Sweat A due to its more acidic pH (4.7),
246 metal(loid) dermal bioaccessibility ranged from 5.1 to 91.0% in SQC001 but it remained
247 lower than 7.2% in soil S9 and lower than 2.9% in soil S8 (Table 3). The overall higher
248 bioaccessibility percentage measured in the reference material can be explained by its lower
249 pH, low total organic carbon content ($< 0.1\%$ w/w) and the fact that the reference material
250 SQC001 has not undergone a natural aging process, which reduces the bioaccessibility of
251 metals in soils. ²⁹

252

253

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255

256

257 **Table 3**

258 Bioaccessibility of As, Cr, Cu, Ni, Pb, and Zn in soils using synthetic sweat formulations A
 259 and B : (a) bioaccessibility expressed in percentage (%) and (b) bioaccessible concentration
 260 (mg l⁻¹)

(a) Bioaccessibility (%)							
Donor solution	Soil	As	Cr	Cu	Ni	Pb	Zn
Sweat A (pH=6.5)	S7	1.4	0.15	0.5	< 0.4	< 0.2	0.05
	S8	0.5	0.06	0.4	< 0.4	< 0.2	0.02
	SQC001	0.06	0.13	2.1	20.4	0.4	40.3
Sweat B (pH=4.7)	S7	2.8	2.0	7.2	0.5	< 0.2	9.0
	S8	0.8	1.0	2.9	0.8	< 0.2	3.0
	SQC001	5.1	25.1	61.3	44.9	34.9	91.0

(b) Bioaccessible concentration (mg l ⁻¹)							
Donor solution	Soil	As	Cr	Cu	Ni	Pb	Zn
Sweat A (pH=6.5)	S7	0.45	0.05	0.38	< 0.01	< 0.01	0.01
	S8	0.76	0.04	0.41	< 0.01	< 0.01	0.01
	SQC001	0.01	0.02	0.17	2.29	0.09	20.6
Sweat B (pH=4.7)	S7	0.86	0.76	5.93	0.01	< 0.01	2.34
	S8	1.36	0.59	3.13	0.02	< 0.01	0.66
	SQC001	0.88	3.12	5.07	5.02	9.20	46.6

261

262

263 In summary, bioaccessibility percentages in terms of sweat are in the following order Sweat
 264 B > Sweat A and in terms of geological materials they are SQC001 > S7 > S8. In agreement
 265 with bioaccessibility percentages, bioaccessible concentrations were relatively low (below 1
 266 mg l⁻¹) in sweat A except for Ni (2.29 mg l⁻¹) and Zn (20.6 mg l⁻¹). The highest bioaccessible
 267 concentrations were measured in SQC001 for Ni, Pb, and Zn. Even though Cu
 268 bioaccessibility percentages following extraction with the more acidic sweat B were only 7.2
 269 and 2.9 % in soils S7 and S8 respectively, high bioaccessible concentrations (5.9 and 3.1 mg
 270 l⁻¹ respectively) were obtained because of the very high Cu content (largely exceeding the C
 271 criterion) measured in these field-collected soil samples (Table 2).

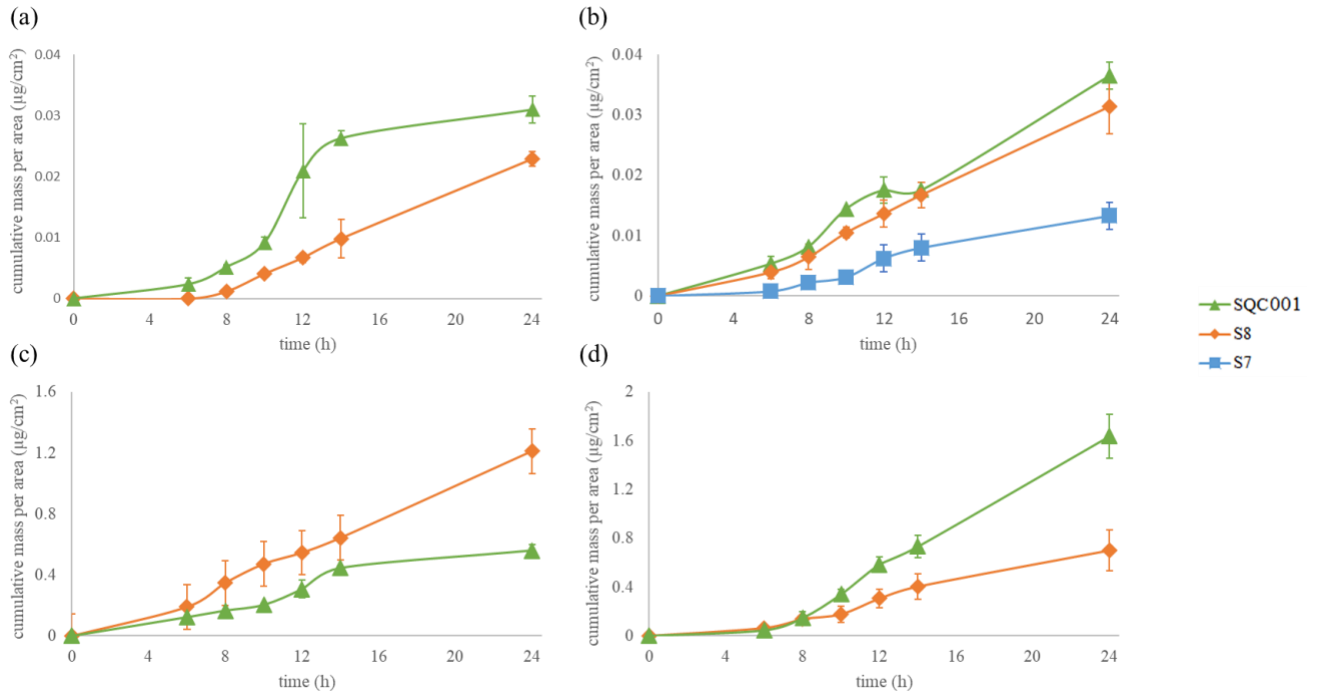
272 A more comprehensive analysis of the influence of soil properties and synthetic sweat
273 formulation on bioaccessibility of metal(loid)s from soils can be found in Leal et al. ⁷, and
274 Marin Villegas et al. ⁸

275 **3.3 Permeation test**

276 Following the bioaccessibility test, the supernatant containing the bioaccessible fraction of
277 metal(loid)s was used as the donor solution in permeation tests. The concentration of
278 metal(loid)s in the receptor ($\mu\text{g l}^{-1}$) was converted to the total metal(loid) amount that
279 permeated ($\mu\text{g.cm}^{-2}$) and then plotted against time (Figure 1 and Figure 2). Flux ($\mu\text{g cm}^{-2} \text{h}^{-1}$)
280 ¹) was calculated as the slope from the steady-state region of graphs shown on Figures 1 and
281 2 and lag time as the intercept of the curve with the X-axis (time). The permeation coefficient
282 K_p was calculated from the linear steady-state region of the plot by dividing the flux through
283 the membrane ($\mu\text{g cm}^{-2} \text{h}^{-1}$) by the concentration in the donor solution. ³⁰

284 Overall, mass balance recovery calculated from donor solutions, synthetic membranes, and
285 receptor solutions yielded satisfactory percentages. Results for all analyzed samples ranged
286 from 58.1 to 101.3 % for Cr and from 85.3 to 128.4 % for Cu (when Sweat A was used as a
287 donor solution) and from 81.9 to 115.2 % for Cr and 73.4 to 101.2 % for Cu when Sweat B
288 was used as a donor solution.

289



290

291 **Figure 1**

292 Mean cumulative mass per area and standard deviation of metal in Sweat A (pH = 6.5) that
 293 permeated through Strat-M membrane for (a) chromium (not coated with sebum), (b)
 294 chromium (coated with sebum), (c) copper (not coated with sebum), and (d) copper (coated
 295 with sebum).

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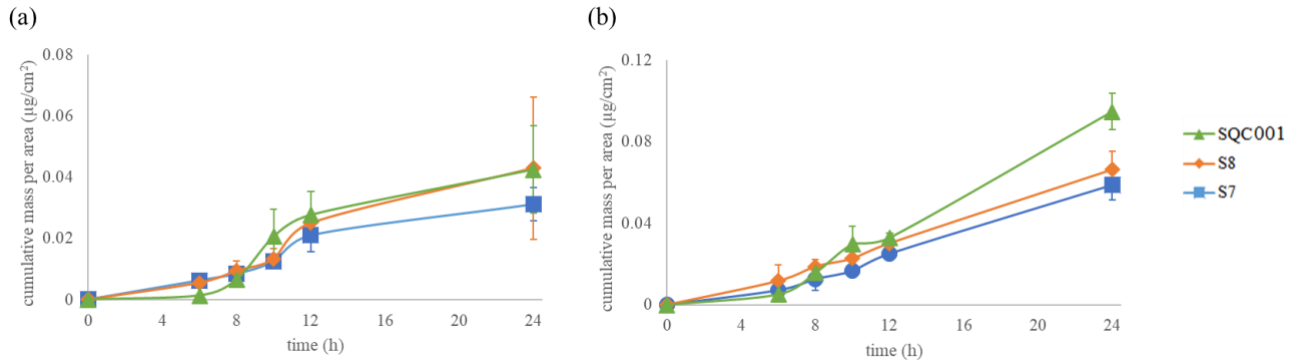
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304 **Figure 2**

305 Mean cumulative mass per area and standard deviation of chromium in Sweat B (pH = 4.7)
 306 that permeated through Strat-M membrane when (a) not coated with sebum and (b) coated
 307 with sebum.

308

309 **3.3.1 Arsenic**

310 Arsenic did not permeate the synthetic skin membrane for all sweat formulation and soil
 311 sample tested. At the pH of both sweats and under oxidizing conditions, As is expected to be
 312 found as As(V), the less mobile form of this metalloid. ³¹⁻³² Experimental data indicate that
 313 penetration of metal(loid)s through the skin is significantly dependent on ion mobility and
 314 charge. ³³

315 **3.3.2 Chromium**

316 Figure 1 and Figure 2 show that Cr permeated through the membrane for both synthetic sweat
 317 formulations and all geological materials (except for soil S7 in Sweat A, in the absence of
 318 sebum). Results (Table 4) indicate that K_p is higher for Sweat A than Sweat B for the same
 319 geological material. This can be explained by the Cr (III) and Cr(VI) species in each sweat
 320 formulation. In the more alkaline Sweat A, and under oxidizing conditions (donor
 321 compartment open to air), a higher amount of Cr is expected to be found as Cr(VI) when

322 compared to the more acidic Sweat B. Cr(VI) has a higher potential of permeation than
323 Cr(III) and a higher associated K_p ^{10, 34, 35}, which explains the higher Flux and K_p of Cr in
324 Sweat A.

325 For Sweat B, flux and K_p are roughly doubled in the presence of sebum for all the geological
326 materials. Sebum might interact with Cr species in sweat B and foment production of more
327 penetrable compounds. In Sweat A, in addition to Cr(VI), Cr(III) species that are expected to
328 be found are mostly $Cr(OH)_2^+$ and neutral thus potentially more permeable $Cr(OH)_3(aq)$,
329 whereas in Sweat B due to a more acidic pH, Cr(III) is expected to be the main Cr oxidation
330 state.³⁶ This hypothesis is backed by the fact that for Sweat A, there is no visible influence
331 of sebum in the permeation parameters. However, other variables such as molecular size,
332 chemical reactivity, and counterions could affect permeation of Cr through the skin
333 membrane^{33, 37} To validate this hypothesis, it is recommended to assess chromium speciation
334 in SSFLs.

335 The permeation coefficient (K_p) also varies among geological materials within the same
336 sweat composition. This might be partially explained by the dose factor: the rate of diffusion
337 of Cr is not proportionate to the applied concentration, absolute absorption can reach a
338 plateau value and then decrease with an increment in concentration.³⁷ This phenomenon
339 happens in real skin due to the buildup of a secondary diffusion barrier as a consequence of
340 electrophilic metals (such as Cr(III)) forming stable bonds with proteins in the skin, also
341 causing longer lag times.³⁷ As previously reported,⁸ the concentrations of metals in the
342 donor solution following dermal bioaccessibility tests are dependent on the physicochemical
343 properties of the geological material and extractant (synthetic sweat). There was a much

344 higher bioaccessible concentration of Cr in Sweat B than Sweat A following bioaccessibility
 345 test (Table 3) producing different diffusion profiles (Figure 1 and Figure 2).

346 **Table 4**

347 Flux ($\text{g cm}^{-2} \text{h}^{-1}$), lag time (h), and permeation coefficient (cm h^{-1}) for (a) Cr and (b) Cu (data
 348 expressed as mean \pm standard deviation).

(a)		Donor solution	Flux ($\text{g cm}^{-2} \text{h}^{-1}$)	Lag Time (h)	Kp (cm h^{-1})
Sweat A (pH=6.5)	S7	-	-	-	-
	S8	1.4E-03 \pm 4.0E-05	6.7 \pm 0.3	3.8E-02 \pm 1.1E-03	
	SQC001	1.7E-03 \pm 4.1E-04	2.8 \pm 0.6	1.1E-01 \pm 2.6E-02	
	S7 Sebum	7.1E-04 \pm 6.5E-05	4.4 \pm 0.1	1.3E-01 \pm 1.2E-02	
	S8 Sebum	1.5E-03 \pm 3.4E-05	5.5 \pm 0.5	4.7E-02 \pm 1.0E-03	
	SQC001 Sebum	1.7E-03 \pm 1.1E-04	2.5 \pm 0.2	1.1E-01 \pm 7.2E-03	
Sweat B (pH=4.7)	S7	1.4E-03 \pm 2.4E-04	0.6 \pm 0.3	1.5E-03 \pm 2.6E-04	
	S8	2.1E-03 \pm 2.6E-04	2.8 \pm 0.4	2.6E-03 \pm 3.3E-04	
	SQC001	2.2E-03 \pm 5.0E-04	2.9 \pm 0.7	7.3E-04 \pm 1.7E-04	
	S7 Sebum	2.9E-03 \pm 2.9E-03	3.7 \pm 0.1	3.2E-03 \pm 3.2E-03	
	S8 Sebum	3.0E-03 \pm 6.3E-05	1.9 \pm 0.1	3.7E-03 \pm 7.8E-05	
	SQC001 Sebum	4.9E-03 \pm 2.0E-04	4.7 \pm 0.3	1.9E-03 \pm 7.7E-05	
(b)		Donor solution	Flux ($\text{g cm}^{-2} \text{h}^{-1}$)	Lag Time (h)	Kp (cm h^{-1})
Sweat A (pH=6.5)	S7	-	-	-	
	S8	5.5E-02 \pm 1.8E-03	0.2 \pm 0.4	1.3E-01 \pm 4.3E-03	
	SQC001	2.6E-02 \pm 4.2E-03	0.6 \pm 0.6	1.5E-01 \pm 2.4E-02	
	S7 Sebum	-	-	-	
	S8 Sebum	3.6E-02 \pm 2.3E-03	4.6 \pm 0.3	8.8E-02 \pm 5.5E-03	
	SQC001 Sebum	9.1E-02 \pm 2.5E-03	5.9 \pm 0.3	5.2E-01 \pm 1.4E-02	

349

350

351 3.3.3 Copper

352 Copper extracted from samples S8 (0.41 mg l^{-1}) and SQC001 (0.17 mg l^{-1}) with Sweat A
 353 permeated the synthetic skin membrane, both in the presence and absence of sebum ((Figure
 354 1 and Table 4). It should be noted that Cu was also detected in the receptor solution following

355 permeation with sample S7 extracted with Sweat A (0.38 mg l^{-1}) in the presence of sebum at
356 the end of the sampling period ($t=12\text{h}$ and $t=24\text{h}$). However, there was not enough data to
357 build the cumulative mass per area versus time curve and calculate the permeation
358 parameters. Copper did not permeate the membrane when extracted with sweat B even
359 though bioaccessible concentrations (donor solution) were much higher (3.13 mg l^{-1} for S8
360 and 5.07 mg l^{-1} for SQC001). This suggests that Cu speciation and complexation, which
361 depends on pH, and sweat formulation among other parameters, might influence the
362 permeation of Cu.

363 The K_p for Cu was similar for soils S8 and SQC001 in the absence of sebum. However, in
364 the presence of sebum, the K_p was around five times higher for SQC001. Lag times before
365 Cu permeation were longer in the presence of sebum for S8 and SQC001, increasing from
366 0.2 to 4.6 h and from 0.6 to 5.9 h , respectively. This could be caused by the added layer
367 formed by the sebum, causing the Cu flux to take a longer time to reach equilibrium.

368 **3.3.4 Nickel**

369 High bioaccessible Ni concentrations ($2.29\text{-}5.02 \text{ mg l}^{-1}$) were obtained when extracting
370 SQC001 with both sweat formulations but low to below detection ($< 0.01 \text{ mg l}^{-1}$)
371 bioaccessible Ni concentrations were measured when extracting field-collected soil samples
372 S7 and S8 (Table 3). In all cases, Ni did not permeate the synthetic skin membrane.
373 Depending on Ni activity, Ni in solutions with a $\text{pH} < 8$ is expected to be mostly found as
374 Ni^{2+} .³⁸ Fullerton et al.³⁹ reported a strong influence of the vehicle in the permeation of NiCl_2
375 and NiSO_4 through the skin and lag times of 50 h (our permeation test lasted 24 h). In another
376 study, Larese et al.³⁰, tested permeation through human abdominal skin of Ni powder in
377 suspension in synthetic sweat (50 g l^{-1}) at $\text{pH} 6.5$ (Sweat A). In the latter study, Ni slowly

378 permeated the skin with a lag phase of 14 h and Ni was present as a free ion in the donor and
379 in the receptor solution. Nevertheless, in the present study, the potential for very long lag
380 times made Ni unable to penetrate the synthetic skin membrane under the experimental
381 design used.

382 **3.3.5 Lead**

383 Lead was only present in donor solutions when SQC001 was extracted with Sweat A (0.09
384 mg l⁻¹) and Sweat B (9.20 mg l⁻¹) (Table 3). However, it did not permeate the synthetic skin
385 membrane. In real human skin, Pb is mainly absorbed through the sweat glands and hair
386 follicles and only slightly mobile through the transepidermal route depending on its
387 speciation.^{10, 40} The Strat-M membrane is designed to specifically simulate the diffusion
388 pathway.

389 **3.3.6 Zinc**

390 Zinc did not permeate the synthetic skin membrane even if very high bioaccessible
391 concentrations (Table 3) were found in the donor solution for both SSFLs with SQC001. Zinc
392 is expected to be mostly present in its charged ionic form Zn²⁺ from low to neutral pH.⁴¹
393 Most of the previous investigations regarding permeation of Zn were focused on ZnO from
394 sunscreen, concluding that particles formed micron-sized aggregates reducing permeation
395 through human skin.^{10,42,43}

396 **3.3.7 Comparison of K_p values with published values**

397 The USEPA¹⁹ recommends K_p values (cm hr⁻¹) for some metals (Cr(III): 0.001, Cr(VI):
398 0.002, Ni:0.0002, Pb:0.0001, Zn: 0.0006, and other non-specified inorganics: 0.001). These
399 values have been adapted from Hostýnek et al.⁴⁴ Because of its conservative approach,

400 USEPA¹⁹ listed the highest reported permeability coefficient. Nonetheless, other studies
401 reported higher permeability coefficients than the ones recommended by USEPA.¹⁹
402 Examples of these are Filon et al.⁴⁵, who reported a K_p for Cr of 0.0124, and Fullerton et al.³⁹,
403 who reported K_p for Ni of 0.0015 for epidermis and 0.23 for the dermis.

404 The K_p from our experiments using the Strat-M synthetic membrane when metal(loid)s
405 extracted in sweat A were used in the donor solution were higher than the ones summarized
406 by USEPA.¹⁹ However the values were in the same order of magnitude or slightly higher for
407 Cr when sweat B was used in the donor solution. Moreover, in the present study, longer lag
408 times and differences in the K_p were found in the presence of sebum. The percutaneous data
409 obtained using a synthetic skin membrane in this study gives valuable insights regarding the
410 influence of the donor solution pH and the presence of sebum.

411 **4. Future research and study limitations**

412 The dermal bioaccessibility of all studied metal(loid)s from geological materials was higher
413 at lower pH. Nevertheless, only Cr and Cu could permeate the Strat-M membrane following
414 bioaccessibility test in synthetic sweat, and Cr and Cu seem to have a greater potential for
415 diffusion through human skin surrogate (Strat-M membrane) at higher pH. For this reason,
416 the characteristics of the donor solution (synthetic sweat formulation) are critical for both
417 bioaccessibility and permeation of metal(loid)s. Further studies are needed to assess
418 metal(loid) speciation in various synthetic SSFLs to help explain differences in bioaccessible
419 metal(loid)s ability to permeate the skin. Moreover, the present study findings also warrant
420 additional studies on the influence of sebum in the permeation of metal(loid)s from various
421 soil types through human skin surrogates.

422 The diffusion of metal(loid)s through a barrier is a complex phenomenon because several
423 factors are interrelated, such as pH, oxidation state, presence of counter ions, dose, and
424 solubility. For risk assessment, environmental agencies like USEPA often suggest
425 standardized or generic values for K_p . However, K_p values are not only metal specific but
426 appear to be site-specific and depend on several variables that must be considered for a more
427 accurate estimation of risk related with dermal exposure.

428 The results obtained in this study showed that membrane Strat-M is suited for early stages of
429 dermal permeation studies of metal(loid)s from contaminated geological materials. Synthetic
430 membranes are commonly available, produce less variability, and are significantly less
431 expensive than human skin. However, the Strat-M membrane only models the diffusion of
432 chemicals and is not suitable to model other dermal pathways such as appendages (through
433 sweat glands and hair follicles). An additional shortcoming of the Strat-M membrane is that
434 it does probably not allow to simulate reservoirs of metal(loids) in the stratum corneum and
435 other layers of the skin. Further research to compare diffusion results with real human skin
436 is therefore necessary to reduce uncertainty in the calculation of dermal exposure to
437 metal(loid)s present in contaminated soils.

438

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445 **List of Tables**

446 **Table 1** Composition of artificial SSFLs.

447 **Table 2** Total concentrations of As, Cr, Cu, Ni, Pb, and Zn (mg kg^{-1}), pH, total organic
448 carbon (TOC, w/w %), and cation exchange capacity (CEC, $\text{meq } 100 \text{ g}^{-1}$) of soils. Precision
449 expressed as \pm relative standard deviation % .

450 **Table 3** Bioaccessibility of As, Cr, Cu, Ni, Pb, and Zn in soils using synthetic sweat
451 formulations A, and B : (a) bioaccessibility expressed in percentage (%) and (b) bioaccessible
452 concentration (mg l^{-1}).

453 **Table 4** Flux ($\text{g cm}^{-2} \text{ h}^{-1}$), lag time (h), and permeation coefficient (cm h^{-1}) for (a) Cr and (b)
454 Cu (data expressed as mean \pm standard deviation).

455

456

457 **List of Figures**

458 **Figure 1** Mean cumulative mass per area and standard deviation of metal in Sweat A (pH =
459 6.5) that permeated through Strat-M membrane for (a) chromium (not coated with sebum),
460 (b) chromium (coated with sebum), (c) copper (not coated with sebum), and (d) copper
461 (coated with sebum).

462 **Figure 2** Mean cumulative mass per area and standard deviation of chromium in Sweat B
463 (pH = 4.7) that permeated through Strat-M membrane when (a) not coated with sebum and
464 (b) coated with sebum.

465

466 **5. References**

- 467 (1) Guney, M., Bourges, C. M. J., Chapuis, R. P., & Zagury, G. J. (2017). Lung
468 bioaccessibility of As, Cu, Fe, Mn, Ni, Pb, and Zn in fine fraction (< 20 µm) from
469 contaminated soils and mine tailings. *Science of the Total Environment*, 579, 378-386.
- 470 (2) Gosselin, M., & Zagury, G. J. (2020). Metal(loid)s inhalation bioaccessibility and
471 oxidative potential of particulate matter from chromated copper arsenate (CCA)-
472 contaminated soils. *Chemosphere*, 238, 124557. doi:10.1016/j.chemosphere.2019.124557
- 473 (3) Van der Kallen, C. C., Gosselin, M., & Zagury, G. J. (2020). Oral and inhalation
474 bioaccessibility of metal(loid)s in chromated copper arsenate (CCA)-contaminated soils:
475 Assessment of particle size influence. *Science of The Total Environment*, 734, 139412.
476 doi:<https://doi.org/10.1016/j.scitotenv.2020.139412>
- 477 (4) Kastury, F., Karna, R. R., Scheckel, K. G., & Juhasz, A. L. (2020). Correlation between
478 lead speciation and inhalation bioaccessibility using two different simulated lung fluids.
479 *Environmental Pollution*, 263, 114609. doi:<https://doi.org/10.1016/j.envpol.2020.114609>
- 480 (5) Stefaniak, A. B., Duling, M. G., Geer, L., & Virji, M. A. (2014). Dissolution of the metal
481 sensitizers Ni, Be, Cr in artificial sweat to improve estimates of dermal bioaccessibility.
482 *Environmental Science Processes & Impacts*, 16, 341-351. doi:10.1039/c3em00570d
- 483 (6) Beriro, D. J., Cave, M. R., Wragg, J., Thomas, R., Wills, G., & Evans, F. (2016). A review
484 of the current state of the art of physiologically-based tests for measuring human dermal in
485 vitro bioavailability of polycyclic aromatic hydrocarbons (PAH) in soil. *Journal of*
486 *Hazardous Materials*, 305, 240-259.

- 487 (7) Leal, L. T. C., Guney, M., & Zagury, G. J. (2018). In vitro dermal bioaccessibility of
488 selected metals in contaminated soil and mine tailings and human health risk characterization.
489 *Chemosphere*, 197, 42-49. doi:10.1016/j.chemosphere.2018.01.008
- 490 (8) Marin Villegas, C. A., Guney, M., & Zagury, G. J. (2019). Comparison of five artificial
491 skin surface film liquids for assessing dermal bioaccessibility of metals in certified reference
492 soils. *Science of The Total Environment*, 692, 595-601.
- 493 (9) Sartorelli, P., Andersen, H.R., Angerer, J., Corish, J., Drexler, H., Göen, T., Griffin, P.,
494 Hotchkiss, S.A.M., Larese, F., Montomoli, L., Perkins, J., Schmelz, M., van de Sandt, J., &
495 Williams, J. (2000). Percutaneous penetration studies for risk assessment. *Environmental*
496 *Toxicology and Pharmacology*, 8, 133-152.
- 497 (10) Franken, A., Eloff, F. C., Du Plessis, J., & Du Plessis, J. L. (2015a). In vitro permeation
498 of metals through human skin: a review and recommendations. *Chemical Research in*
499 *Toxicology*, 28, 2237-2249.
- 500 (11) Sartorelli, P., Montomoli, L., & Sisinni, A. (2012). Percutaneous penetration of metals
501 and their effects on skin. *Prevention & Research*, 2, 158-164.
- 502 (12) Franz, T. J. (1975). Percutaneous absorption. On the relevance of in vitro data. *Journal*
503 *of Investigative Dermatology*, 64, 190-195.
- 504 (13) Haq, A., Dorrani, M., Goodyear, B., Joshi, V., & Michniak-Kohn, B. (2018a).
505 Membrane properties for permeability testing: Skin versus synthetic membranes.
506 *International Journal of Pharmaceutics*, 539, 58-64. doi:10.1016/j.ijpharm.2018.01.029

507 (14) Moody, R. P., Nadeau, B., & Chu, I. (1995). In vivo and in vitro dermal absorption of
508 benzo [a] pyrene in rat, guinea pig, human and tissue-cultured skin. *Journal of*
509 *Dermatological Science*, 9, 48-58.

510 (15) Barbero, A. M., & Frasch, H. F. (2009). Pig and guinea pig skin as surrogates for human
511 in vitro penetration studies: a quantitative review. *Toxicology in Vitro*, 23, 1-13.

512 (16) Haq, A., Goodyear, B., Ameen, D., Joshi, V., & Michniak-Kohn, B. (2018b). Strat-M(R)
513 synthetic membrane: Permeability comparison to human cadaver skin. *International Journal*
514 *of Pharmaceutics*, 547, 432-437. doi:10.1016/j.ijpharm.2018.06.012

515 (17) Joshi, V., Brewster, D., & Colonero, P. (2012). In vitro diffusion studies in transdermal
516 research: a synthetic membrane model in place of human skin. *Drug Development and*
517 *Delivery*, 12, 40-42.

518 (18) Hillwalker, W. E., & Anderson, K. A. (2014). Bioaccessibility of metals in alloys:
519 evaluation of three surrogate biofluids. *Environmental Pollution*, 185, 52-58.
520 doi:10.1016/j.envpol.2013.10.006

521 (19) USEPA. (2004). Risk Assessment Guidance for Superfund Volume I: Human Health
522 Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment):
523 EPA/540/R/99/005. OSWER 9285. 7-02EP PB99-963312, Washington, DC, USA.

524 (20) Clesceri, L. S., Greenberg, A.E., & Eaton, A.D. (1999). Standard Methods for the
525 Examination of Water and Wastewater. American Public Health Association, twentieth
526 edition.

527 (21) ASTM. (2013). D4972-13 Standard Test Method for pH of Soils. West Conshohocken,
528 PA.

529 (22) Tiessen, H., & Moir, J. (1993). Total and Organic Carbon. Soil Sampling and Methods
530 of Analysis. Martin R. Carter (Ed.). Canadian Society of Soil Science: Lewis Publishers.
531 Boca Raton, Florida, USA. pp.

532 (23) Chapman, H. D. (1965). Cation-exchange capacity. Methods of Soil Analysis: Part 2
533 Chemical and Microbiological Properties, 9, 891-901.

534 (24) CEN (2015). Reference test method for release of nickel from all post assemblies which
535 are inserted into pierced parts of the human body and articles intended to come into direct
536 and prolonged contact with the skin. EN 1811: European Committee for Standardization.

537 (25) Wainman, T., Hazen, R. E., & Liroy, P. J. (1994). The extractability of Cr(VI) from
538 contaminated soil in synthetic sweat. Journal of Exposure Analysis and Environmental
539 Epidemiology, 4, 171-181.

540 (26) Wertz, P. W. (2008). Human synthetic sebum formulation and stability under conditions
541 of use and storage. International Journal of Cosmetic Science, 31, 21-25.

542 (27) Franken, A., Eloff, F. C., Du Plessis, J., Badenhorst, C. J., & Du Plessis, J. L. (2015b).
543 In vitro permeation of platinum through African and Caucasian skin. Toxicology Letters,
544 232, 566-572. doi:10.1016/j.toxlet.2014.12.010

545 (28) Ministère de l'Environnement et de la Lutte contre les changements climatiques (2021).
546 Guide d'intervention - Protection des sols et réhabilitation des terrains contaminés, Annexe

547 2 retrieved 26-03-2021 from [https://www.environnement.gouv.qc.ca/sol/terrains/guide-](https://www.environnement.gouv.qc.ca/sol/terrains/guide-intervention/annexe2.pdf)
548 [intervention/annexe2.pdf](https://www.environnement.gouv.qc.ca/sol/terrains/guide-intervention/annexe2.pdf)

549 (29) Liang, S., Guan, D.-X., Li, J., Zhou, C.-Y., Luo, J., & Ma, L. Q. (2016). Effect of aging
550 on bioaccessibility of arsenic and lead in soils. *Chemosphere*, 151, 94-100.
551 doi:<https://doi.org/10.1016/j.chemosphere.2016.02.070>

552 (30) Larese, F., Gianpietro, A., Venier, M., Maina, G., & Renzi, N. (2007). In vitro
553 percutaneous absorption of metal compounds. *Toxicology Letters*, 170, 49-56.
554 doi:<https://doi.org/10.1016/j.toxlet.2007.02.009>

555 (31) McLean, J. E., & Bledsoe, B. E. (1992). Ground water issue: behavior of metals in soils.
556 United States Environmental Protection Agency (EPA/540/S-92/018), Washington.

557 (32) Dobran, S., & Zagury, G. J. (2006). Arsenic speciation and mobilization in CCA-
558 contaminated soils: Influence of organic matter content. *Science of The Total Environment*,
559 364, 239-250.

560 (33) Hostýnek, J. J., Hinz, R. S., Lorence, C. R., Price, M., & Guy, R. H. (1993). Metals and
561 the skin. *Critical Reviews in Toxicology*, 23, 171-235.

562 (34) Gammelgaard, B., Fullerton, A., Avnstorp, C., & Menné, T. (1992). Permeation of
563 chromium salts through human skin in vitro. *Contact Dermatitis*, 27, 302-310.

564 (35) Van Lierde, V., Chery, C. C., Moens, L., & Vanhaecke, F. (2005). Capillary
565 electrophoresis hyphenated to inductively coupled plasma-sector field-mass spectrometry for
566 the detection of chromium species after incubation of chromium in simulated sweat.
567 *Electrophoresis*, 26, 1703-1711. doi:10.1002/elps.200410221

568 (36) World Health Organization. (2009). Inorganic chromium (III) compounds. World
569 Health Organization.

570 (37) Hostýnek, J.J. (2003). Factors determining percutaneous metal absorption. Food and
571 Chemical Toxicology, 41, 327-345.

572 (38) Gonsalves, L., Marinov, S., Gryglewicz, G., Carleer, R., & Yperman, J. (2016).
573 Preparation, characterization and application of polystyrene based activated carbons for Ni
574 (II) removal from aqueous solution. Fuel Processing Technology, 149, 75-85.

575 (39) Fullerton, A., Andersen, J., & Hoelgaard, A. (1988). Permeation of nickel through
576 human skin in vitro—effect of vehicles. British Journal of Dermatology, 118, 509-516.

577 (40) Stauber, J., Florence, T., Gulson, B., & Dale, L. (1994). Percutaneous absorption of
578 inorganic lead compounds. Science of The Total Environment, 145, 55-70.

579 (41) Powell, K. J., Brown, P. L., Byrne, R. H., Gajda, T., Hefter, G., Leuz, A.K., Sjöberg,
580 Staffan., & Wanner, H. (2015). Chemical Speciation of Environmentally Significant Metals:
581 An IUPAC contribution to reliable and rigorous computer modelling. Chemistry
582 International, 37, 15-19.

583 (42) Mavon, A., Miquel, C., Lejeune, O., Payre, B., & Moretto, P. (2007). In vitro
584 percutaneous absorption and in vivo stratum corneum distribution of an organic and a mineral
585 sunscreen. Skin Pharmacology and Physiology, 20, 10-20.

586 (43) Merwe, D. v. d., Tawde, S., Pickrell, J. A., & Erickson, L. E. (2009). Nanocrystalline
587 titanium dioxide and magnesium oxide in vitro dermal absorption in human skin. Cutaneous
588 and Ocular Toxicology, 28, 78-82.

- 589 (44) Hostýnek, J.J., Hinz, R., Lorence, C., & Guy, R. (1998). Human skin penetration by
590 metal compounds. *Drugs and the Pharmaceutical Sciences*, 91, 647-668.
- 591 (45) Filon, F. L., D'Agostin, F., Crosera, M., Adami, G., Bovenzi, M., & Maina, G. (2008).
592 In vitro percutaneous absorption of chromium powder and the effect of skin cleanser.
593 *Toxicology in Vitro*, 22, 1562-1567.