



Bioaccessibility of nickel and cobalt in powders and massive forms of stainless steel, nickel- or cobalt-based alloys, and nickel and cobalt metals in artificial sweat

Xuying Wang, Gunilla Herting, Zheng Wei, Inger Odnevall Wallinder, Yolanda Hedberg*

KTH Royal Institute of Technology, School of Engineering Sciences in Chemistry, Biotechnology and Health, Department of Chemistry, Division of Surface and Corrosion Science, Drottning Kristinas V. 51, SE-10044, Stockholm, Sweden

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ABSTRACT

Nickel (Ni) and cobalt (Co) are the most common metal allergens upon skin contact at occupational settings and during consumer handling of metals and alloys. A standardized test (EN, 1811) exists to assess Ni release from articles of metals and alloys in massive forms intended for direct and prolonged skin contact, but no corresponding test exists for other materials such as powders or massive forms of alloys placed on the market or to determine the release of Co, for which only limited data is available. Differences in Ni and Co release from massive forms of a range of common stainless steels and some high-alloyed grades compared to Ni and Co metals were therefore assessed in artificial sweat for 1 week at 30 °C according to EN 1811. A comparable modified test procedure was elaborated and used for powders and some selected massive alloys. All alloys investigated released significantly less amount of Ni (100–5000-fold) and Co (200–400,000-fold) compared with Ni and Co metal, respectively. Almost all alloys showed a lower bioaccessible concentration (0.007–6.8 wt% Ni and 0.00003–0.6 wt% Co) when compared to corresponding bulk alloy contents (0.1–53 wt% Ni, 0.02–65 wt% Co). Observed differences are, among other factors, related to differences in bulk composition and to surface oxide characteristics. For the powders, less Ni and Co were released per surface area, but more per mass, compared to the corresponding massive forms.

1. Introduction

Nickel (Ni) and cobalt (Co) are the most common metals causing skin contact allergy, affecting about 10–20% and 1–2% of the general population, respectively (Julander, 2018; Lidén, 2018). Exposure sources are many, both at occupational settings such as handling of metals and alloys during production, processes, and transport, and human use of consumer products such as cell phones and jewelry (Julander, 2018; Lidén, 2018). The Nickel Directive was adopted in 1994 in Europe and contains several sections for different kinds of products and intentional use (EU, 1994). The release restriction limit of Ni is 0.2 µg/cm²/week and 0.5 µg/cm²/week for items inserted into pierced body parts and for items intended to come into direct and prolonged contact with the skin, respectively (EC, 2007). In 2007, the restrictions for Ni in consumer products and articles have been included in entry 27 of Annex XVII of REACH (EC, 2007). Compliance is ensured by testing following the EN 1811 standard (one-week immersion in artificial sweat at 30 °C) for as-received surface conditions of such

articles (CEN, 2015).

The current hazard classification for both Ni and Co metal includes Skin Sens. 1 (may cause an allergic skin reaction). Both Ni and Co are in the same hazard category for skin sensitization (Skin Sens. 1), but in contrast to Ni, there is currently no Co release testing required for items intended for direct and prolonged skin contact.

Available release investigations in artificial sweat for items intended for direct and prolonged skin contact have mostly focused on Ni release for articles and massive surfaces (Erfani et al., 2015; Haudrechy et al., 1993, 1997; Jensen et al., 2003; Kettelarij et al., 2014; Lidén et al., 2008; Midander et al., 2016; Uter and Wolter, 2018), whereas only few studies exist on Co release (Julander et al., 2009; Kettelarij et al., 2014; Midander et al., 2014, 2016; Uter and Wolter, 2018) and on Ni or Co release from powders (Hedberg et al., 2016; Mazinanian et al., 2013; Midander et al., 2007). Powders may be deposited on the skin during different occupational work tasks such as grinding.

The objectives of this study were to i) provide bioaccessibility data on Ni and Co for stainless steels and other high-alloyed grades of

* Corresponding author.

E-mail address: yolanda@kth.se (Y. Hedberg).

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Table 1

Nominal bulk alloy composition (wt.%, based on supplier information) of materials (massive forms) tested following the EN 1811 method.

Grade	EN number	C (wt.%)	Mn (wt.%)	Ni (wt.%)	Cr (wt.%)	Mo (wt.%)	S (wt.%)	Co (wt.%)	Fe (wt.%)	Supplier
FeCr11	1.4003	0.017	0.60	0.38	11	0.0080	0.012	0.015	bal.	Aperam
430	1.4016	0.034	0.32	0.11	16	0.015	0.0016	0.019	bal.	Aperam
FeCr18	1.4509	0.014	0.34	0.21	18	0.027	0.0090	0.026	bal.	Aperam
FeCr21	1.4622	0.02	0.41	0.30	21	0.040	< 0.0005	0.020	bal.	Outokumpu
630	1.4542	0.017	0.80	4.5	15	0.15	0.020	0.13	bal.	UGITECH
304	1.4301	0.039	1.2	9.0	18	0.36	0.0029	0.16	bal.	Aperam
303	1.4305	0.057	1.7	8.3	17	0.39	0.29	0.146	bal.	UGITECH
LDX2101	1.4162	0.023	4.8	1.6	21	0.28	0.0010	0.039	bal.	Outokumpu
FeCr23Ni3	1.4062	0.018	1.3	2.6	23	0.22	0.0080	0.068	bal.	Aperam
316LVM	1.4441	0.022	1.8	15	18	2.7	0.0008	0.048	bal.	UGITECH
FeCr20Ni9	1.4420	0.017	1.8	8.5	20	0.64	0.0010	0.27	bal.	Outokumpu
316L (a)	1.4404	0.019	0.90	10	17	2	< 0.0005	0.20	bal.	Outokumpu
316L (b)	1.4404 m.g.	0.018	1.3	10	17	2.1	0.025	0.12	bal.	UGITECH
316L (c)	1.4435	0.021	1.8	13	17	2.6	0.014	0.095	bal.	UGITECH
FeCr21Ni11	1.4472	0.039	4.0	11	21	2.1	0.0005	0.033	bal.	UGITECH
IN718	2.4668	< 0.08	N/A	53	18	3.0	N/A	0.030	18	UGITECH
R30008	2.4711	0.061	1.9	16	20	7.2	0.0010	39	16	UGITECH
Alloy 605	2.4964	N/A	N/A	11	20	N/A	N/A	50	bal.	UGITECH
CoCr28Mo6	CoCr	N/A	N/A	0.21	28	N/A	N/A	65	N/A	UGITECH

N/A – no data available; bal. – balance; C – carbon; Mn – manganese; Ni – nickel; Cr – chromium; Mo – molybdenum; S – sulfur; Co – cobalt; Fe – iron.

different surface composition and passivity compared with the pure metals in artificial sweat according to EN 1811; *ii*) elaborate a modified test procedure to enable reproducible testing of massive sheets independent of transport and storage history; *iii*) elaborate a modified test procedure applicable for powders; and *iv*) compare released amounts of Ni and Co for selected alloys in massive form and as fine sized (< 5 µm) powders.

2. Materials and methods

2.1. Materials, chemicals and solutions

A list of the alloys (massive forms) investigated by the EN 1811:2011 + A1:2015 (CEN, 2015) standard method is shown in Table 1 together with their nominal bulk composition (based on supplier information): in total 15 stainless steels [rectangle - FeCr11, 430, FeCr18, FeCr21, 304, LDX2101, FeCr23Ni3, FeCr20Ni9; cylinder - 316LVM, 303, 630, 316L (two suppliers and two grades - noted as three letters a, b and c), FeCr21Ni11] and four high-alloyed steels (cylinder - IN718, Alloy 605, CoCr28Mo6; disk - R30008).

Four stainless steels [rectangle - 304 (a), 316L (d), 430 (a), LDX2101 (a)], Co metal and Ni metal in their massive forms were selected for further testing using a modified EN 1811 procedure (section 2.2.), Table 2. The letters (a) and (d) after each grade name in Table 2 are used to distinguish the different massive coupons of similar grades in Table 1. Testing was also performed for the same alloys and metals as powders except for LDX2101 (which was not available in powder form).

Table 2

Nominal bulk composition (wt.%, based on supplier information) of test items (massives, and powders - p) investigated following the modified EN 1811 method.

Grade	EN number	C (wt%)	Mn (wt%)	Ni (wt%)	Cr (wt%)	Mo (wt%)	S (wt%)	Co (wt%)	Fe (wt%)	Supplier
304 (a)	1.4301	0.038	1.2	9.0	18	0.36	0.0029	0.16	bal.	Aperam
316L (d)	1.4404	0.017	1.3	10	17	2.0	0.00060	0.24	bal.	Aperam
430 (a)	1.4016	0.034	0.32	0.11	16	0.015	0.0016	0.019	bal.	Aperam
LDX2101 (a)	1.4162	0.023	4.8	1.6	21	0.28	0.0010	0.039	bal.	Outokumpu
Ni	Ni	N/A	N/A	99.995	N/A	N/A	N/A	< 0.0001	N/A	Nipera
Co	Co	N/A	N/A	0.018	N/A	N/A	N/A	99.9	N/A	ECTX
304-p	1.4301	0.02	1.44	9.4	18.8	N/A	0.08	0.07	69.6	Sandvik Osprey
316L-p	1.4404	N/A	0.97	10.5	16.6	2.2	0.004	0.06	69	Sandvik Osprey
430-p	1.4016	0.02	0.52	0.11	17.1	N/A	0.004	0.03	81.8	Sandvik Osprey
IN625-p	2.4856	0.01	0.24	63.68	21.2	9.1	0.006	0.01	2.2	Sandvik Osprey
Ni-p	Ni	N/A	N/A	99.999	N/A	N/A	N/A	0.01196	N/A	Nipera
Co-p	Co	0.033	0.0017	0.0027	N/A	N/A	0.0009	98.7	N/A	ECTX

N/A – no data available; bal. – balance; C – carbon; Mn – manganese; Ni – nickel; Cr – chromium; Mo – molybdenum; S – sulfur; Co – cobalt; Fe – iron.

condition. A modified test protocol was also applied on selected massive grades and powders for two reasons: i) to enable comparison of massive surfaces with powders, and ii) to eliminate the effect of sample history (storage, surface finish, surface treatments) when comparing different alloys and metals. As-received surfaces can differ largely in terms of storage between manufacture and testing, conditions that influence the surface oxide characteristics (e.g. composition, thickness, passivity) and hence the metal release process (Hedberg and Odneval Wallinder, 2016). Testing of as-received surfaces of products on the market is highly relevant for legislation, but less relevant for a fair and accurate comparison between different alloy grades and metals. A modified surface preparation procedure was therefore elaborated with a defined grinding, ageing, and passivation procedure that, as closely as possible, simulates as-received surface conditions and ensures equal test conditions for all test items (Hedberg et al., 2013; Herting et al., 2005, 2008; Mazinanian et al., 2016). In this modified test procedure, the test items were after the grinding procedure air aged at dry conditions and room temperature for 24 h followed by 2 h passivation in artificial sweat at 30 °C, to mimic as-received (passive) surfaces. This procedure was the same for the alloy and metal massive surfaces, hence enabling their comparison. Differences between as-received surfaces and surfaces aged via this procedure were evaluated. The EN 1811 method does not stipulate any use of solution agitation during the one-week exposure period, an approach found to be reproducible for massive surfaces. However, gentle agitation is required for testing of powders to avoid extensive particle aggregation and poor reproducibility (Hedberg and Odneval Wallinder, 2012; Midander et al., 2006; Midander et al., 2007; OECD, 2001). We therefore employed a gentle well-defined agitation procedure for the modified test procedure for both powders and massive surfaces with the aim to enable comparison between massive and powder forms with a maximized surface area for the powders. This gentle agitation hinders agglomeration without damaging the powder surface by wear processes. Differences and similarities between the EN 1811 and the modified EN 1811 method are schematically illustrated in Fig. 1. Detailed procedures are described in the following subsections.

2.2.1. Materials preparation

All grades listed in Table 1 were investigated following the standardized EN 1811 method. Testing was also performed on a reference alloy (76 wt% Au, 16 wt% Cu, 6 wt% Ni, 2 wt% Zn) as stipulated by the EN 1811 protocol. Surface cleaning was performed by gentle shaking (100 rpm) in ultrapure water and neutral detergent (Extran, VWR, Sweden) for 2 min at room temperature, followed by rinsing with ultrapure water and drying using nitrogen gas at room temperature. The test items were then placed in a desiccator at room temperature for 24 ± 1 h (ageing) prior to the immersion testing, shown in Fig. 1.

For the massive materials in the modified test procedure, all surfaces were abraded with 1200 grit SiC paper, cleaned ultrasonically consecutively in acetone and ethanol for 5 min and dried with nitrogen gas at room temperature. This was followed by ageing for 24 h in a desiccator at room temperature prior to passivation for 2 h in artificial sweat at 30 °C, rinsing with ultrapure water and direct immersion in artificial sweat for one week (30 °C, gentle bilinear agitation - 12° inclination, 22 cycles per min) at dark conditions. As grinding of the powders is impossible, they were not pre-passivated but investigated with their as-received surface conditions. 5 ± 0.5 mg of the dry powder was weighed with a microbalance with 2 µg readability (XP26 DeltaRange®, METTLER TOLEDO, Sweden) in an acid-cleaned dry Nalgene® polypropylene vessel. The exact weight was documented.

2.2.2. Exposure in artificial sweat for one week

For each test item, parallel triplicate coupons and one or two blank samples (without any test items), were exposed in parallel at 30 ± 2 °C (dark conditions, closed vessels for 168 ± 2 h) in an incubator (Edmund Bühler GmbH TH30, Bodelshausen, Germany). All massive forms were exposed at a surface area to solution volume ratio of 1 cm²/

mL as stipulated in the EN 1811 standard (CEN, 2015) and all powders at a mass to solution volume ratio of 0.1 g/L (Hedberg and Odneval Wallinder, 2012). After exposure, or after solid-liquid separation in the case of the powders, the solutions were acidified with 65% HNO₃ until pH < 2 to prevent deposition of released metals to the interior surface of the containers as stipulated by the EN 1811 protocol, before stored at ambient laboratory conditions (for 1–2 weeks) until analysis.

2.2.2.1. EN 1811 method. The EN1811 method describes exposure of massive surfaces in non-agitated solution. After the immersion period, the test items were carefully removed from the solution, rinsed with ultrapure water, dried with nitrogen gas at room temperature, and stored in the desiccator for further surface analysis using X-ray photoelectron spectroscopy (XPS), while the artificial sweat solutions were transferred to new acid-cleaned vessels to be acidified with 65% HNO₃ to pH < 2 for metal analysis.

2.2.2.2. Modified EN 1811 method. For the massive surfaces, the immersion procedures were similar as described in the EN 1811 protocol except for different surface preparation (section 2.2.1) and the use of gentle agitation (bilinear agitation - 12° inclination, 22 cycles per min bilinear). For the powders, 50 mL solution was added to 5 mg powder sample (0.1 g/L loading). After the immersion period, 7.5 mL of the test solution (including powder particles) was transferred from the upper part of the solution to a 15 mL centrifuge tube and centrifuged at 3000 relative centrifugal force (rcf, g) for 10 min to separate powder particles from the test solution. After the centrifuging process, 6.5 mL of the particle-free supernatant was transferred to a new acid-cleaned vessel and acidified with 65% HNO₃ to a pH less than 2 for metal analysis.

2.2.3. Atomic absorption spectroscopy and data evaluation

Total concentrations of released Ni and Co from the test items into artificial sweat were analyzed by means of graphite furnace (GF-) or flame atomic absorption spectroscopy (AAS) using a PerkinElmer AA800 analyst instrument and triplicate readings of each sample. The calibration curves were based on at least four standard calibration points. The measured and presented sample concentrations were all above the limits of detection (1.55–1.59 µg/L Ni or Co), calculated based on three times the maximum standard deviation of all blank samples, and below the highest calibration point. If sample concentrations were below the lowest obtainable limit of detection (LOD), this is denoted < LOD in the figures and tables. All blank concentrations were lower than 0.60 µg/L Ni and 0.46 µg/L Co, when analyzed with the most sensitive method (GF-AAS) of the two methods used. If the sample concentration was outside the calibration range, it was re-analyzed diluted or using another calibration range. Quality control standards of known concentrations (QC) were analyzed every 4–6th sample and showed acceptable concentrations (QC ± 15%QC). Certified standard samples for Ni and Co were analyzed showing recoveries of 102% for both metals.

Average sample concentrations for three independent triplicate samples are presented with the corresponding blank concentration(s) subtracted. Released amounts of metals expressed as µg/cm²/week or µg/cm² correspond to the blank-corrected concentration of released metals (µg/L) normalized to the solution volume (L) and the exposed surface area. The geometric area of the massive samples was determined individually by measuring their lengths, widths, thicknesses, diameter, and/or heights using a caliper. All surface areas are provided in Tables S1–2, supplementary material. The exposed surface area is defined as the geometrical surface area for the massive materials and as the hydrodynamic surface area determined in artificial sweat using static light scattering (see section 2.3) for the powders. Data presented as µg/g is based on measured released concentrations multiplied by the solution volume (L) and divided by the initial powder sample weight (g).

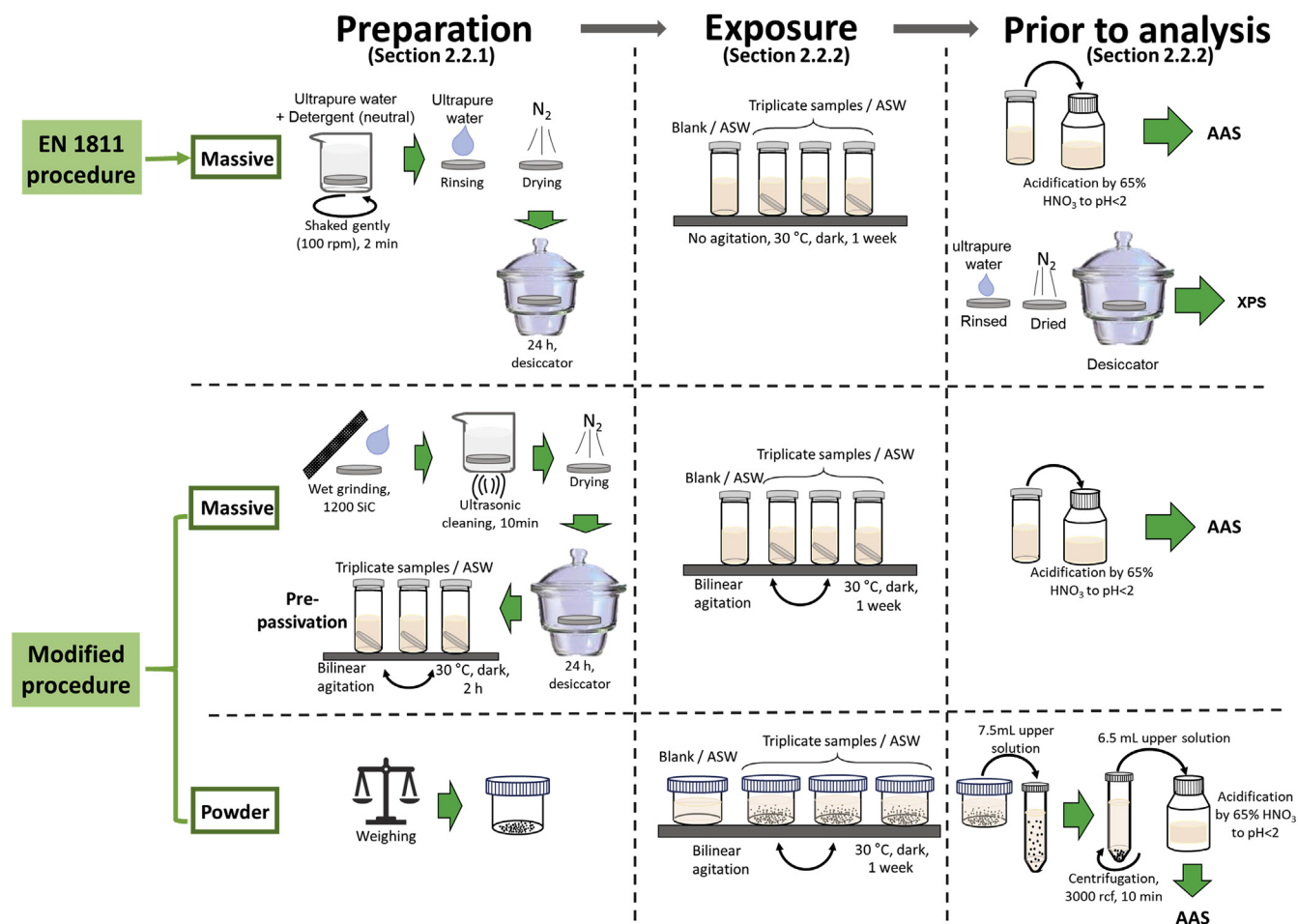


Fig. 1. Experimental schemes as stipulated in EN 1811 and as elaborated in this study for the modified test procedure. ASW - artificial sweat, XPS - x-ray photoelectron spectroscopy, AAS - atomic absorption spectroscopy.

2.3. Static light scattering

For powder characterization in artificial sweat, triplicate measurements of the particle size distribution in artificial sweat (prepared according to EN 1811) were performed using a static light scattering technique using a Malvern Mastersizer 3000 instrument. The particle size was plotted against the volume percentage. Refractive indexes of alloys (2.757), Co (2.1396), Ni (1.958) and water (1.33) (since it was the solvent of the test medium), as well as the particle density, were used as input parameters applying standard operational conditions calculating the hydrodynamic surface area (SSA) directly in the instrument software according to eq. (1).

$$SSA = \frac{6 \sum \frac{V_i}{d_i}}{p \sum V_i} \quad (1)$$

Where V_i is the relative volume in class i with mean class diameter of d_i , and p is the particle effective density. Spherical shape is assumed for this specific surface area determination. For the alloy powders, the input density was chosen as the same as for pristine particles (no agglomeration accounted for), since the particle size observed in artificial sweat was similar as the size measured using scanning electron microscopy: 304 (7.8 g/cm³), 316L (7.9 g/cm³), 430 (7.8 g/cm³), and IN625 (8.44 g/cm³). For the Ni powder, an effective density of 7.33 g/cm³ was chosen as input value, corresponding to about 80 vol% density of Ni in the agglomerates with the remaining 20 vol% being water. For the Co powder, the most prone powder to agglomerate in this study, an effective density of 4.93 g/cm³ was chosen as input parameter,

corresponding to 50 vol% Co and 50 vol% water in the agglomerates. Despite these uncertainties and possible sources of error, the hydrodynamic specific surface area is preferred as compared to the dry specific surface area, as it accounts for solution-induced agglomeration of the particles and is a better estimation of the specific surface area in artificial sweat.

2.4. Scanning electron microscopy

The shape and morphology of all investigated powders were prior to exposure characterized by means of Scanning Electron Microscopy (FEI XL30 SEM and INCA software) at 20 kV up to 100,000 times magnification using secondary electrons. The powders were fixed on carbon tape. Their bulk composition was also confirmed by measurements of energy dispersive X-ray spectroscopy.

2.5. Brunauer Emmett Teller method

The specific surface area (m²/g) of the powders at dry conditions was determined by means of the Brunauer Emmett Teller (BET) technique using a Micromeritics Gemini V instrument that adsorbs nitrogen gas at cryogenic condition. As the gas adsorption is pressure-dependent, the specific surface area of powder particles is determined by knowing the gas pressure and the adsorbed gas volume. All measurements were conducted at Sandvik Heating Technology AB, Sweden.

2.6. X-ray photoelectron spectroscopy

Compositional analysis of the outermost surface (approx. 5–10 nm) of selected as-received alloys tested by the EN 1811 method was conducted for unexposed and exposed alloy test items by X-ray photoelectron spectroscopy (XPS, UltraDLD spectrometer, Kratos Analytical, monochromatic 150W Al X-ray source on areas sized $700 \times 300 \mu\text{m}^2$). High resolution spectra (20 eV pass energy) were acquired for Cr 2p, Ni 2p, Fe 2p, Mn 2p, O 1s, using C1s as energy reference (285.0 eV). The results are presented as the relative mass content of oxidized Fe, Cr, Ni and Mn in the outermost surface oxide, e.g. $[\text{Cr}_{\text{ox}}/(\text{Cr}_{\text{ox}} + \text{Fe}_{\text{ox}} + \text{Ni}_{\text{ox}} + \text{Mn}_{\text{ox}})]$. Relative changes in surface oxide thickness (before and after exposure) were roughly assessed based on the relative mass content ratio of oxide to metal signals.

2.7. Open circuit potential measurements

In order to investigate the corrosion tendency of the test items in artificial sweat, changes in open circuit potential (OCP) with time were determined for triplicate massive coupons of grades 303 and 316L (d) (surface area: 0.79 cm^2) in artificial sweat for 1 week at room temperature using an Ag/AgCl saturated KCl reference electrode. An increasing and relatively high OCP means generally, at these conditions, high corrosion resistance and a stabilizing surface layer with time. Prior to the measurements, the coupons were ground with 1200 SiC paper, cleaned ultrasonically in acetone and ethanol for 5 min each, and dried with nitrogen gas at room temperature, followed by ageing for one week in a desiccator at room temperature. All measurements were performed using a PARSTAT MC Multichannel Potentiostat (Solartron Analytical) equipped with the VersaStudio software.

2.8. Statistical significance methods

An unpaired Student's t-test with unequal variance (KaleidaGraph v. 4.0) was applied to determine the statistical significance of differences between two independent sets of samples. When $p < 0.05$, the difference was considered statistically significant and denoted with asterisks in the figures (* for $p < 0.05$ and ** for $p < 0.01$).

3. Results and discussion

3.1. Ni release from the different alloys using the EN 1811 method

Testing of the reference material resulted in a Ni release rate of $0.27 \pm 0.002 \mu\text{g}/\text{cm}^2/\text{week}$, well within the range of $0.19\text{--}0.49 \mu\text{g}/\text{cm}^2/\text{week}$ stipulated by the EN 1811 protocol. These results ensure the validity of generated test results of this study. Released amount ($\mu\text{g}/\text{cm}^2/\text{week}$) of Ni after one week from the as-received massive alloys are presented in Fig. 2 (a), and their release as a function of the Ni bulk content (wt.%) of each alloy is presented in Fig. 2 (b). All alloys released Ni less than $0.5 \mu\text{g}/\text{cm}^2/\text{week}$, i.e. below the restriction limit set by the Nickel Directive for the release of Ni from items intended to come into direct and prolonged contact with the skin (EC, 2007). The combined measurement uncertainty of the EN 1811 standard has been determined to 46%, which means that items releasing less than $0.88 \mu\text{g Ni}/\text{cm}^2/\text{week}$ comply with the EU nickel restriction (CEN, 2015). For piercing items, the limit is set to $0.2 \mu\text{g Ni}/\text{cm}^2/\text{week}$ (EC, 2007) (when considering the uncertainty, $0.35 \mu\text{g}/\text{cm}^2/\text{week}$ (CEN, 2015)). Two grades generate results between these two limits, grade 303 ($0.29 \mu\text{g}/\text{cm}^2/\text{week}$) and IN718 ($0.30 \mu\text{g}/\text{cm}^2/\text{week}$) but below the recently determined uncertainty-based limit of $0.35 \mu\text{g}/\text{cm}^2/\text{week}$ for pierced items (CEN, 2015). The intended use of these alloys is though not for such applications. Results of Fig. 2 (a) clearly show that most alloy grades (in particular stainless steels) release very low amounts of Ni into the test solution, much lower (about 5–100-fold) than the lower restriction limit ($0.2 \mu\text{g}/\text{cm}^2/\text{week}$). As shown in Fig. 2 (b), the bulk

composition cannot fully explain the Ni release pattern. There is nevertheless a trend with increasing amounts of released metal with increasing bulk content, e.g. evident for Ni release from IN718 (53 wt% Ni). For the stainless steels investigated (0.11–13 wt% Ni), the release of Ni seems mainly to be influenced by the surface characteristics, for instance evident for grade 303 that is a sulfur-enriched alloy forming Mn-sulfide inclusions at the surface (Sedriks, 1996), while the bulk composition does not seem to influence the results. Release data of Ni from massive Ni and Co metals, exposed using the modified EN 1811 method, are included in Fig. 2 for comparison. The tested alloys released 100–5000-fold less Ni as compared to Ni metal.

3.2. Co release from the different alloys using the EN 1811 method

There is at present no requirement to test the Co release from items intended to come in prolonged or frequent skin contact. Due to the lack of a relevant method, the EN1811 method was used to determine the Co release in artificial sweat.

Released amount ($\mu\text{g}/\text{cm}^2/\text{week}$) of Co after one week from the as-received massive alloys are presented in Fig. 3 (a), as well as their release as a function of the Co bulk content (wt.%) of each alloy in Fig. 3 (b). Released amounts were very low ($< 0.01 \mu\text{g}/\text{cm}^2/\text{week}$) for most alloys, except the Co-based alloys R300008 (39 wt% Co), Alloy 605 (50 wt% Co) and CoCr28Mo6 (63 wt% Co) with high Co bulk content.

The tested alloys released 200–400,000-fold less Co as compared to Co metal, Fig. 3.

3.3. Bioaccessible concentration and relative bioaccessibility

Table 3 presents the bioaccessible concentration of released Ni and Co from all alloys calculated as the concentration of the released metal (normalized on surface area) from the alloy compared with corresponding release of the same metal from the pure metal exposed at identical conditions (eq. (2)). It also shows the relative bioaccessibility of released Ni and Co, as determined by eq. (3).

Bioaccessible concentration

$$= \frac{\text{Released concentration alloy}_{\text{Co or Ni}} \left(\frac{\mu\text{g}}{\text{cm}^2} \right)}{\text{Released concentration pure metal}_{\text{Co or Ni}} \left(\frac{\mu\text{g}}{\text{cm}^2} \right)} \times 100\% \quad (2)$$

$$\text{Relative bioaccessibility} = \frac{\text{Released amount alloy}_{\text{Co or Ni}} \left(\frac{\mu\text{g}}{\text{cm}^2} \right)}{\text{Released amount pure metal}_{\text{Co or Ni}} \left(\frac{\mu\text{g}}{\text{cm}^2} \right)} \times \frac{\text{Bulk content pure metal}_{\text{Co or Ni}} (\text{wt}\%)}{\text{Bulk content alloy}_{\text{Co or Ni}} (\text{wt}\%)} \times 100\% \quad (3)$$

In this study, all alloys generally released lower amounts of both Ni and Co than expected compared with the pure metals and when compared with their Ni or Co bulk alloy contents. A similar trend is observed when the bioaccessible concentration and relative bioaccessibility are calculated based on normalization on mass, Table S3 in supplementary material. However, the normalization on mass is less relevant in this study as the specific surface area varied among the test coupons, in contrast to the surface area to solution volume ratio which was kept constant. All alloys, except for the FeCr11 alloy due to a large variation for Ni release, showed a relative bioaccessibility being significantly less than 1, Fig. 2 (meaning lower metal release per bulk alloy content as compared with the behavior of the pure metal), see Tables 3 and S3. All alloys exhibited mainly lower bioaccessible concentrations as compared to their bulk alloy contents, a behavior that most probably is related to their beneficial surface passivity (Hedberg et al., 2016). When comparing metals with alloys, massive alloys were compared with massive metals, Tables 3 and S3. However, differences in specific surface area among the grades and metals still could compromise a fair comparison based on mass (Table S3), which due to a relatively massive

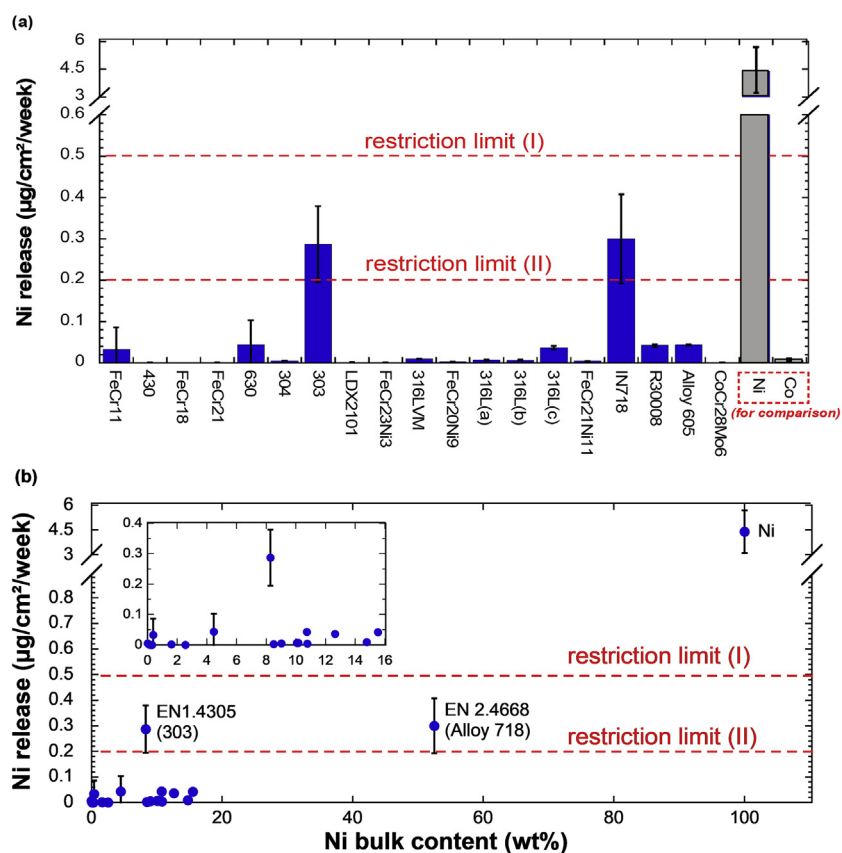


Fig. 2. (a) Released amount of Ni from 19 as-received massive alloys exposed for one week in artificial sweat following the EN 1811 protocol, and (b) their release as a function of Ni bulk content. Results for Ni and Co metals, investigated according to the modified EN 1811 method, are included for comparison. Error bars show the standard deviation of three independent coupons. The restriction limits of 0.2 and 0.5 μg Ni/ cm^2/week are defined in entry 27 of Annex XVII of REACH (EC, 2007). Corresponding raw data is given in Table S1, supplementary material.

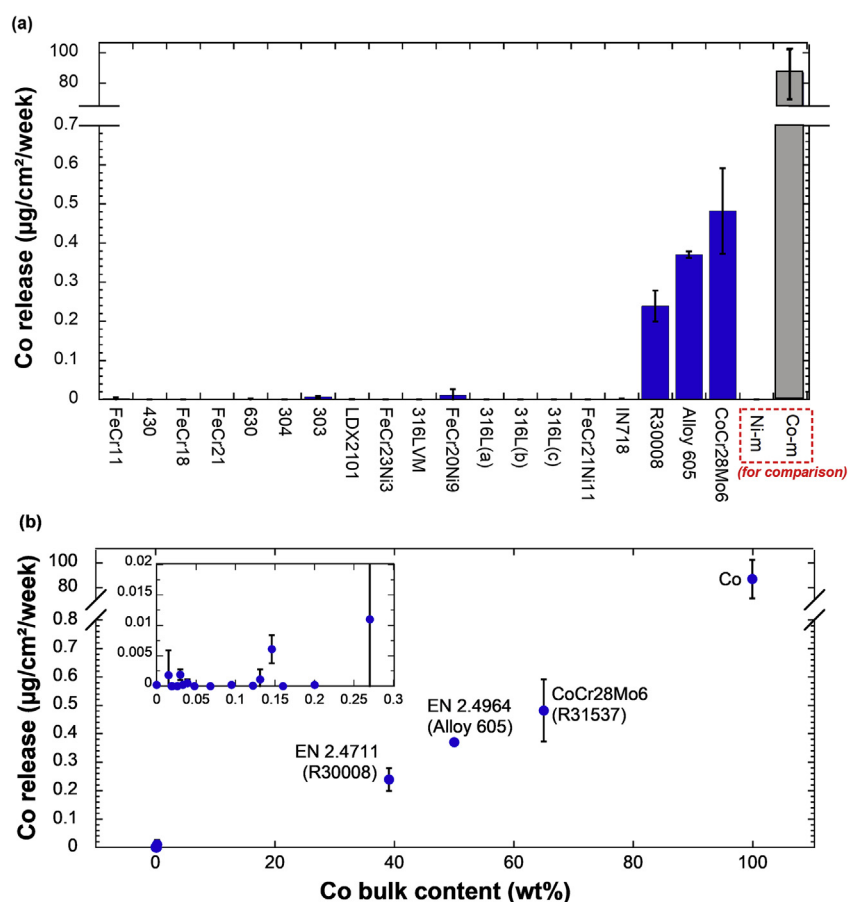


Fig. 3. (a) Released amount of Co from 19 as-received massive alloys exposed for one week in artificial sweat following the EN 1811 protocol, and (b) their release as a function of Co bulk content. The error bars show the standard deviation of three independent coupons. Corresponding raw data is given in Table S1, supplementary material.

Table 3

Bulk composition, bioaccessible concentration, and relative bioaccessibility of Ni and Co for the different as-received massive alloys exposed to artificial sweat for one week following the EN 1811 standard method. Data is calculated by the normalization on surface area. Corresponding data normalized on mass is shown in Table S3, supplementary material. Results for Ni and Co metals are included for comparison (a 100% bioaccessible concentration and a relative bioaccessibility of 1 for the pure metals per definition, Eqs. (2) and (3)).

Grade	EN number	wt.% Ni		Relative bioaccessibility of Ni	wt.% Co		Relative bioaccessibility of Co
		Bulk	Bioaccessible concentration		Bulk	Bioaccessible concentration	
FeCr11	1.4003	0.38	0.76	2.0	0.015	0.0025	0.17
430	1.4016	0.11	0.016	0.14	0.019	0.00015	0.0079
FeCr18	1.4509	0.21	< LOD	< LOD	0.026	0.00018	0.0071
FeCr21	1.4622	0.3	0.0068	0.023	0.02	< LOD	< LOD
630	1.4542	4.5	0.98	0.22	0.13	0.0013	0.0097
304	1.4301	9	0.12	0.013	0.16	0.000069	0.00040
303	1.4305	8.3	6.5	0.79	0.146	0.0070	0.048
LDX2101	1.4162	1.6	0.034	0.021	0.039	0.00058	0.015
FeCr23Ni3	1.4062	2.6	0.0068	0.0026	0.068	0.00015	0.0022
316LVM	1.4441	15	0.21	0.014	0.048	0.000034	0.00070
FeCr20Ni9	1.4420	8.5	0.059	0.0070	0.27	0.013	0.047
316L (a)	1.4404	10	0.16	0.016	0.2	0.00023	0.0011
316L (b)	1.4404 m g.	10	0.15	0.015	0.12	0.00012	0.0010
316L (c)	1.4435	13	0.83	0.064	0.095	0.00023	0.0024
FeCr21Ni11	1.4472	11	0.10	0.0093	0.033	0.00023	0.0070
IN718	2.4668	53	6.8	0.13	0.03	0.0022	0.073
R30008	2.4711	16	0.96	0.060	39	0.28	0.0070
Alloy 605	2.4964	11	0.98	0.089	50	0.43	0.0085
CoCr28Mo6	CoCr	0.21	0.0091	0.043	65	0.55	0.0085
Ni	Ni	99.995	100	1	< 0.0001	0.00023	> 2.3
Co	Co	0.0183	0.13	6.8	99.9	100	1

< LOD – released metal concentrations below limits of detection.

Co metal slab (large mass and small surface area) tended to result in a higher relative bioaccessibility of alloys when normalized on mass (Table S3) as compared with the bioaccessibility normalized on surface area (Table 3). In the case of Co, 316L(a) showed for example a 3-fold higher relative bioaccessibility when normalized on mass as compared to when normalized on surface area, since the difference in specific surface area of the flat 316L coupon and the massive Co metal slab was large. When the bioaccessible concentration is considered as a refinement of bulk concentration for the purpose of classification of alloys based on Co content (RAC, 2017), the calculation based on the metal mass (Eqs. S1-S2, supplementary material) is more appropriate (Table S3). In contrast, in the case of Ni bioaccessibility for massive materials, legislative restrictions are based on the release of Ni per surface area and therefore the relative bioaccessibility based on surface area (Table 3) is more relevant from a legislative perspective.

3.4. Surface composition and passivity

The relative oxidized metal composition (determined by means of XPS) of the outermost surface of selected alloys prior to, and after exposure to artificial sweat for one week, are presented in Fig. 4a. The surface oxide of all investigated stainless steels consists of Fe- and Cr-oxides, but no Ni oxides. Some alloys contain oxidized Mn (Mn 2p_{3/2}: 641.2 ± 0.02 eV). The IN718 surface oxide also contains oxidized Ni (Ni 2p_{3/2}: 855.5 ± 0.3 eV and 856.2 ± 0.1 eV before and after exposure), Fig. 4a. For all other alloys, Ni was only observed in its metallic form (Ni 2p_{3/2}: 853.3 ± 0.6 eV), which coincides with its expected enrichment beneath the surface oxide (Hanawa et al., 2001; Hedberg and Odneval Wallinder, 2016). In all cases, oxidized Cr was observed in its trivalent form (Cr 2p_{3/2}: 577.4 ± 0.9 eV). It is evident that Cr is enriched in the surface oxide as compared to the bulk content, already for the as-received surfaces prior to exposure, and even more after exposure to artificial sweat, Fig. 4a. This is expected for chromium-rich corrosion-resistant alloys (Hedberg and Odneval Wallinder, 2016). Metallic peaks of Fe (Fe 2p_{3/2}: 707.6 ± 0.8 eV) and Cr (Cr 2p_{3/2}: 574.5 ± 0.5 eV) were observed for all surfaces with reduced intensity compared with the oxidized peaks upon exposure to artificial sweat,

Fig. 4b. This implies a thickening of the surface oxide with time in artificial sweat. The most significant effect was observed for the 303 and 430 stainless steels, the least corrosion-resistant grades among the investigated alloys.

Changes in electrochemical activity with time were investigated for massive surfaces of the least and the most corrosion-resistant austenitic stainless steel grades, 303 and 316L (d), as these grades showed large differences in Ni release (Fig. 2). The grades differ mostly in sulfur content, but not largely in bulk content of Cr, Fe, or Ni. Changes in OCP with time are shown in Fig. 5. The results show a more noble (positive) OCP for 316L (d) compared with 303 without any features corresponding to metastable pitting corrosion, whereas 303 reveals a less positive OCP and a curve that is interrupted by a number of features indicative of metastable pitting events, Fig. 5. This means a higher passivity and oxide stability of the 316L (d) grade as compared with 303. The OCP curves were highly reproducible, Fig. S2 in supplementary material. The high sulfur content (0.29 wt%) in 303 is correlated to the presence of MnS-inclusions at the metal surface (Sedriks, 1996), which is furthermore supported by the presence of Mn in the outermost surface oxide, Fig. 4. These inclusions facilitate the induction of metastable pitting in artificial sweat and that most probably are responsible for the relatively high Ni release for this grade compared to tested grades of 316L.

3.5. Comparison of test methods for massive surfaces

To enable a comparison between different massive alloys and between a massive versus a powder of a given alloy or metal, effects of different sample history and surface conditions had to be eliminated and solution agitation conducted. These aspects were taken into account in the modified test procedure, see experimental, by using gentle solution agitation and well-defined surface preparation and ageing of the massive surfaces. Differences in results of testing following the EN 1811 standard method and the modified method are illustrated in Fig. 6 for four stainless steel grades (grades given in Tables 1 and 2). Observed differences are relatively small (from 1.2-fold to 5.3-fold) and statistically significant only for Ni release from 304 and Co release from 316L.

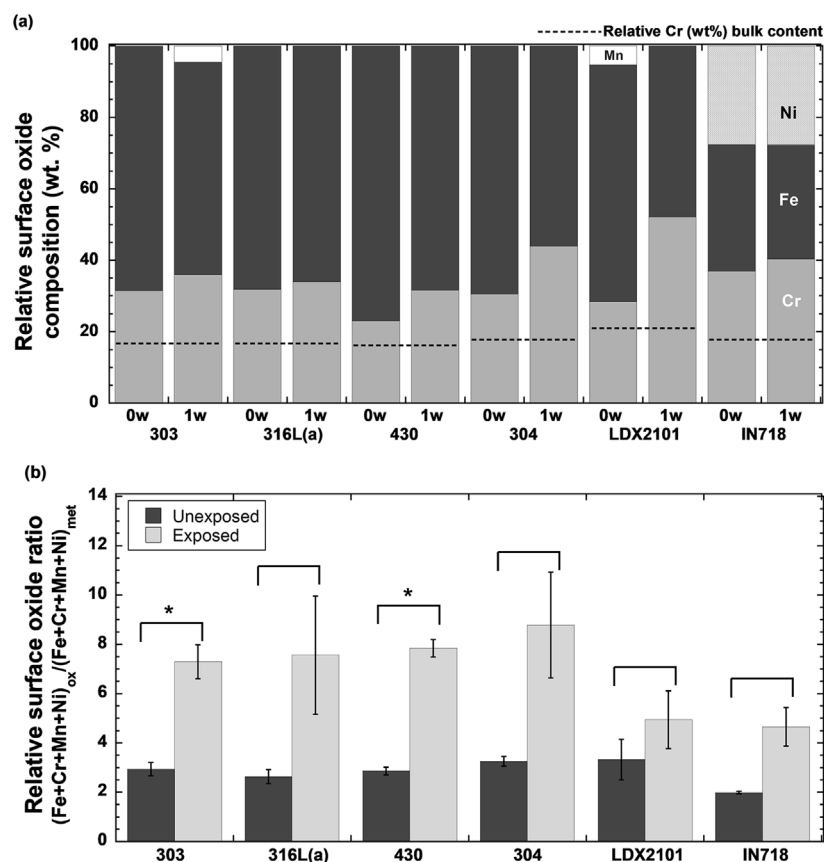


Fig. 4. (a) Relative mass content (wt.%) of oxidized metals (Fe, Cr, Mn and Ni) by means of XPS, and (b) relative ratio (wt.%) of oxidized to metallic peaks of observed metals in the outermost surface oxide of selected massive alloys (stainless steels - 303, 316L(a), 430, 304, LDX2101, nickel-based alloy - IN718), prior to (unexposed - 0w) and after (exposed - 1w) exposure to artificial sweat for one week (168 h) according to the EN 1811 standard procedure. Average values of independent duplicate coupons (each measured twice on different surface locations). Significant differences are shown as * - $p < 0.05$.

The relatively large error bars for Co release from LDX2101 are a result of a variation between independent triplicate coupons and very low concentrations, e.g. two of three coupons in the modified EN 1811 method resulted in a lower Co release as compared with the blank sample concentration of Co, Fig. 6b. The comparison between the modified and the standard EN 1811 suggests that the surface preparation method in the modified protocol results in comparable surfaces as for as-received surface conditions, and that the gentle agitation employed also for massive surfaces did not largely influence the results compared with non-agitated solution conditions.

3.6. Powder characteristics and powders vs. massive surfaces

The powders of this study were all spherical and of comparable sizes, Fig. 7 and Table 4. All alloy particles had similar size both at dry and wet conditions. The Ni and Co metal powders were smaller sized at

dry conditions, but similarly sized compared with the alloy particles at wet conditions due to a larger extent of agglomeration in solution.

When comparing the Ni and Co release from the same grades of different form following the modified EN 1811 method (Table 2), massive versus powder, the release was higher (up to 10-fold) or similar for the massive forms when normalized to the surface area, but lower when normalized to the mass, Fig. 8. From a chemical and material perspective, normalization to surface area is most relevant, as metal release is a surface reaction. However, since the specific surface area of powders might vary in different exposures and is not always easily determined, the normalization on mass is more relevant from a toxicological perspective. The release of less amounts of metals from the powders when normalized on surface area is expected and in accordance with previous findings (Hedberg et al., 2013). The underlying reasons are i) that agglomeration may occur in solution and that the surface area hence not totally is exposed to the solution, a risk

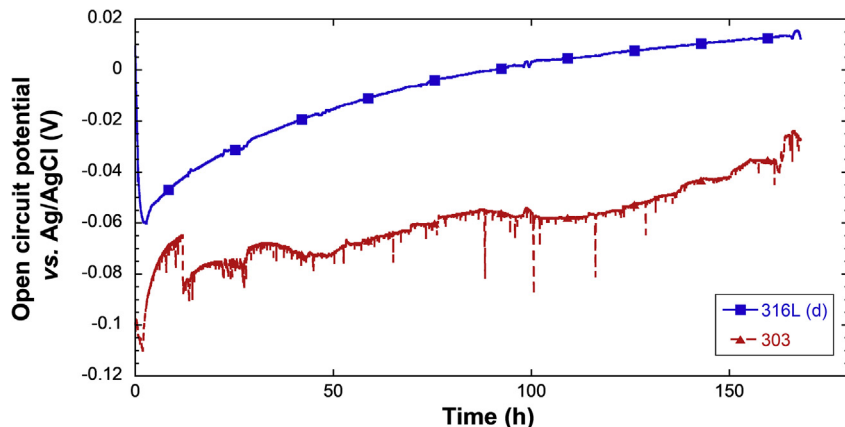


Fig. 5. Changes in open-circuit potential (OCP) with time for one representative sample of massive surfaces of 316L (d) (blue line) and 303 (red line) exposed in artificial sweat (pH 6.55) for 168 h at room temperature. Average OCP curves based on three independent samples are shown for each grade in the supplementary material in Fig. S2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

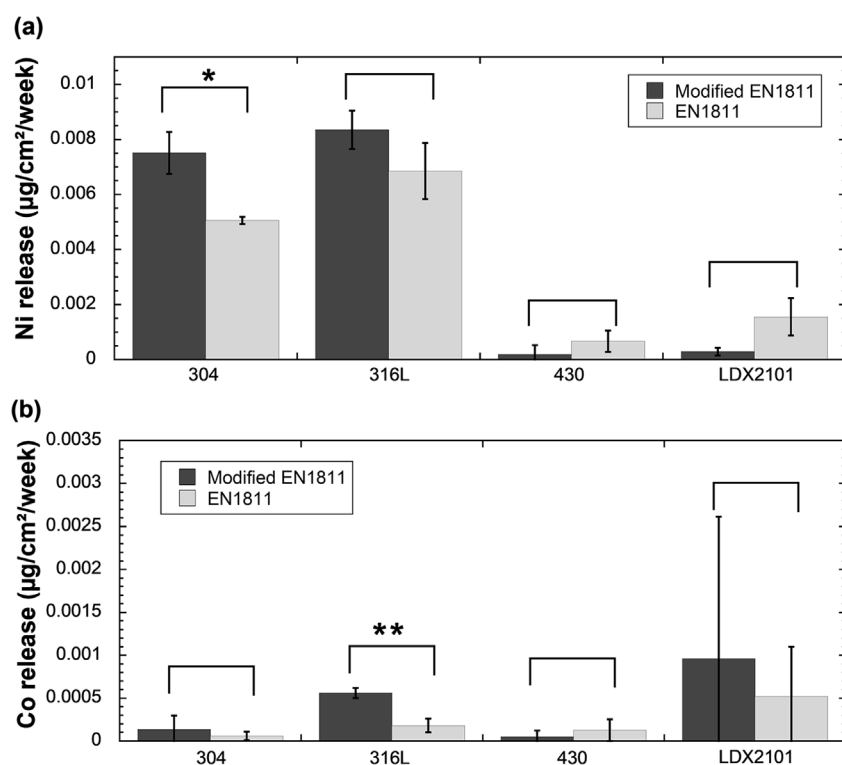


Fig. 6. Comparison between the release of Ni (a) and Co (b) from massive surfaces of stainless steel (grades 304 & 304 (a), 316L (a) & 316L (d), 430 & 430 (a), and LDX2101 & LDX2101 (a)) in artificial sweat for 1 week at 30 °C following the EN 1811 standard protocol and the modified method of this study. Error bars show the standard deviation of three independent coupons. Nominal alloy compositions are given in Tables 1 and 2. Significant differences are shown as * for $p < 0.05$ and ** for $p < 0.01$.

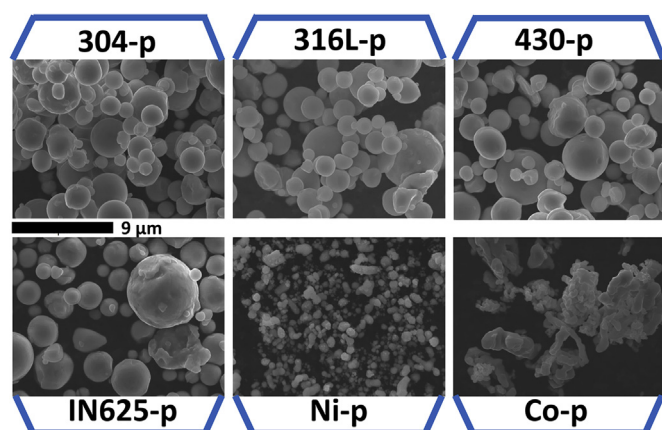


Fig. 7. Scanning electron microscopy (SEM) images of the particle morphology and size at dry conditions for powders of 304, 316L, 430, IN625, Ni metal and Co metal (Table 2) at the same magnification.

Table 4

Specific surface area (m^2/g) of powders at dry conditions based on nitrogen adsorption (BET) and in artificial sweat (static light scattering, Mastersizer). Average values of three measurements are shown with a standard deviation less than 10% in all cases for size distribution cut-off values (μm , 10, 50, and 90% of volume size distribution) in artificial sweat.

Grade	304	316L	430	IN625	Ni	Co
Dry (BET) (m^2/g)	0.39	0.4	0.41	0.33	2.3	1.4
in ASW (Mastersizer) (m^2/g)	0.38	0.32	0.29	0.40	0.79	0.26
10% size (by volume) (μm)	2.1	2.2	2.4	2.0	0.9	2.7
50% size (by volume) (μm)	3.4	3.9	4.2	3.7	1.8	5.4
90% size (by volume) (μm)	5.4	6.7	7.4	6.5	4.5	11.9

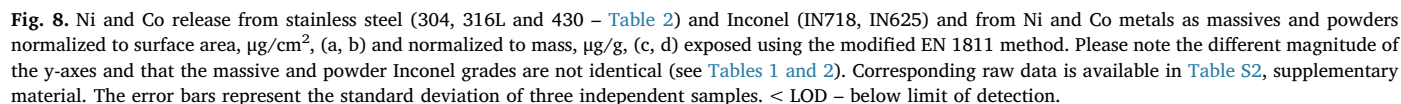
BET – Brunauer Emmet Teller method; ASW – artificial sweat.

minimized in this study by using a 0.1 g/L mass loading and gentle agitation (Hedberg and Odneval Wallinder, 2012; Midander et al., 2006; Midander et al., 2007), and ii) the presence of relatively passive surface oxides of gas-atomized and rapidly cooled fine sized powder particles (Hedberg et al., 2013; Hedberg and Midander, 2014). When normalized to mass, the release of metals from the powders was significantly larger compared with their corresponding massive forms (200–8000-fold). This can be explained by a larger surface area exposed to solution, e.g. 8000-fold larger for 5 mg 316L powder compared with 5 mg massive sheet of 316L. A larger surface area provides a larger possibility of surface reactions and metal release. In general, the Ni and Co release per mass increases with the specific surface area (surface area per mass).

For all alloy powders, the bioaccessible concentration and relative bioaccessibility of released Ni and Co calculated as the concentration of the released metal (normalized on surface area as well as normalized on metal mass) from the alloy compared with corresponding release of the same metal from the pure metal powder exposed at identical conditions (by modified EN, 1811 method) are presented in Tables S4 and S5 in the supplementary material. All alloy powders showed lower bioaccessible concentrations as compared to their bulk contents, as well as lower relative bioaccessibility (significantly less than 1), which is explained by their passive surface properties. For example, when normalized to mass, the bioaccessible concentration of the alloy powders was 0.005–2.1% Ni (as compared to 0.1–64% Ni in the bulk). This corresponds to a relative bioaccessibility of 0.006–0.05, that is, 20–170-fold lower release than expected from the behavior of the pure Ni metal powder and the bulk content.

3.7. Health and regulatory aspects

Ni and Co are skin allergens, and dose-response studies on allergic patients by patch testing and other methods can provide important information on elicitation threshold values for causing allergic contact dermatitis in Ni- or Co-sensitized persons. Elicitation threshold values are relevant for already sensitized individuals. Elicitation threshold values are no restriction limits. Currently, restriction limits exist only



for Ni released from articles and items intended to come into direct and prolonged contact with the skin. The Ni restriction limit is normalized on the surface area and intended for massive surfaces. No guideline exists on how to treat powders with respect to the Nickel Directive. According to recent literature, a skin dose of $0.78 \mu\text{g Ni/cm}^2$ elicits contact dermatitis in 10% of Ni-allergic individuals (ED10) and a skin dose of $10 \mu\text{g Ni/cm}^2$ is the elicitation threshold for 50% of Ni-allergic individuals (ED50) (Fischer et al., 2007; Lidén, 2018). For repeated exposure, the elicitation threshold is lower per application ($0.035 \mu\text{g Ni/cm}^2$) (Fischer et al., 2007). Corresponding elicitation doses of Co are reported in the range of 0.066 – $1.95 \mu\text{g/cm}^2$ for ED10 and 1.45 – $17 \mu\text{g/cm}^2$ for ED50 (Jülander, 2018). Those elicitation limits were derived using doses of fully soluble metal compounds (metal salts, not the metal itself) and are normalized on the area of skin exposed for 48 h to the dose applied (not the surface area of the metal material). For massive metals and alloys, these elicitation threshold levels can to some extent be compared with findings of this study, in which the amount of Ni and Co release from stainless steels were at most $0.043 \mu\text{g Ni/cm}^2$ and $0.011 \mu\text{g Co/cm}^2$ except from grade 303 at most $0.30 \mu\text{g Ni/cm}^2$ and $0.0061 \mu\text{g Co/cm}^2$. The Ni-based alloy released at most $0.30 \mu\text{g Ni/cm}^2$ and $0.0019 \mu\text{g Co/cm}^2$ after one week in artificial sweat. The release of Co from the Co-based alloys was 0.2 – $0.5 \mu\text{g/cm}^2$ after one week of exposure in artificial sweat. This suggests that, for the alloys investigated in this study, the Co-based alloys are most probably of highest concern from a skin allergy perspective regarding Co sensitization, and the stainless steel grade 303 and the Ni-based alloy IN718 of highest concern regarding Ni sensitization. Skin reactions for Ni-sensitized individuals have in previous studies been reported for grade 303, but not for other stainless steel grades (Haudrechy et al., 1993, 1997; Jensen et al., 2003; Summer et al., 2007), which is hence in very good agreement with this study. Regarding powders, a comparison to existing skin elicitation threshold values is even more difficult than for massive surfaces. Assuming the alloy powders would be fully absorbed by the skin at a high concentration (worst case), their release per surface area would in most cases still be lower than from the massive surfaces (Fig. 8). One exception is the IN625 powder, which releases a slightly higher value of Co (though still $< 0.006 \mu\text{g/cm}^2$) as compared to the IN718 massive surface, but still 10-fold lower than the lowest reported Co elicitation threshold value.

4. Conclusions

A comparative study of a range of common stainless steel- and other high-alloyed grades was performed to assess bioaccessibility data on Ni and Co release in artificial sweat according to the EN 1811 standard. The following main conclusions were drawn:

- A modified EN1811 test procedure was elaborated that includes gentle solution agitation and well-defined sample preparation and ageing of massive surfaces in order to eliminate effects of differences in sample history of as-received surfaces. The results were similar to findings for as-received surfaces. These modified test conditions allow a fair comparison between alloys and metals as massives and powders.
- Results generated using the standard EN 1811 protocol and the modified EN 1811 method were comparable in terms of released Ni and Co from the four investigated massive stainless steel grades.
- All alloys investigated released significantly less amounts of Ni (100–5000-fold) compared to the behavior from Ni metal and compared to the restriction limit of $0.5 \mu\text{g/cm}^2/\text{week}$ for items intended to come into direct and prolonged skin contact. With $0.3 \mu\text{g Ni/cm}^2/\text{week}$, the Inconel alloy IN718 and the stainless steel grade 303 were the alloys releasing most Ni in this study. All other alloys in massive or powder form released Ni in amounts that were far below the lower restriction limit ($0.2 \mu\text{g Ni/cm}^2/\text{week}$) for piercing items set by EN 1811.

- For Co release, all alloys investigated released significantly less amounts (200–400,000-fold) compared to the release from Co metal. The Co-based alloys R30008, Alloy 605, and CoCr28Mo6 released most Co (0.2 – $0.5 \mu\text{g/cm}^2/\text{week}$), and the released amounts for all other alloys were very low ($< 0.01 \mu\text{g Co/cm}^2/\text{week}$).
- For most alloys, the bioaccessible concentration levels of Ni and Co were significantly less than the corresponding massive bulk alloy contents, with bioaccessible concentrations (0.007 – $6.8 \text{ wt\% Ni}$ and 0.00003 – $0.6 \text{ wt\% Co}$) considerably lower than corresponding bulk alloy contents (0.1 – $53 \text{ wt\% Ni}$, 0.02 – $65 \text{ wt\% Co}$). A similar behavior was observed for when comparing alloy powders with metal powders.
- The extent of Ni and Co release was related to differences in bulk composition, surface oxide composition and passivity. This was exemplified for the stainless steel grades 316L and 303 with similar Fe, Cr, and Ni alloy composition, but different surface passivity (measured by means of OCP) probably related to the presence of MnS inclusions in 303, as supported by observed Mn oxides on the outermost surface (by XPS) and its high sulfur content ($0.29 \text{ wt\% S}$). Metastable pitting corrosion events were observed in artificial sweat for grade 303, a grade that released most Ni ($0.3 \mu\text{g/cm}^2/\text{week}$) among the investigated stainless steel grades.
- Powders released significantly less or similar Ni and Co per surface area, but significantly more Ni and Co when normalized on mass, as compared to massive surfaces.

Declaration of interests

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.yrtph.2019.04.017>.

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Supplemental Material

Bioaccessibility of nickel and cobalt in powders and massive forms of stainless steel, nickel- or cobalt-based alloys, and nickel and cobalt metals in artificial sweat

Xuying Wang, Gunilla Herting, Zheng Wei, Inger Odnevall Wallinder, and Yolanda Hedberg

Content: Figures S1-S2, Tables S1-S5

Figure S1 shows the XPS spectra of Fe 2p and Cr 2p on the sample 304 prior to (unexposed) and after (exposed) exposure in artificial sweat for one week exposure according to the non-modified EN 1811 standard protocol.

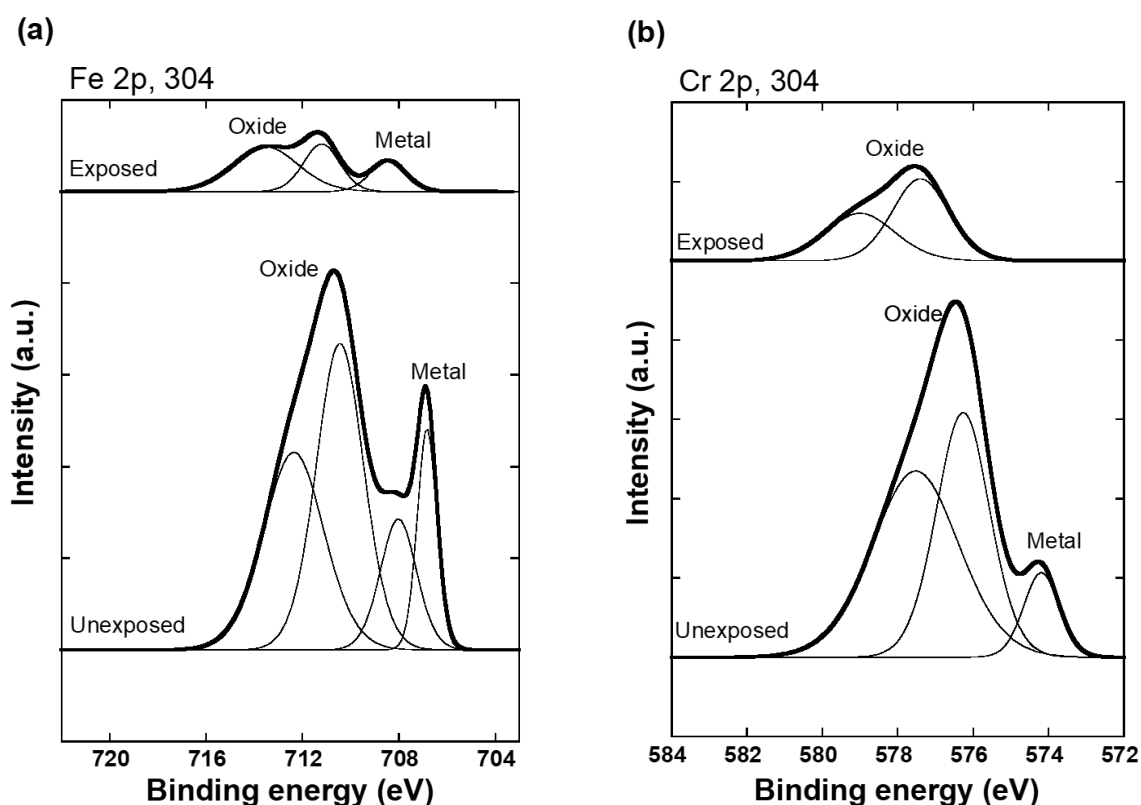


Figure S1. XPS spectra of (a) Fe 2p and (b) Cr 2p on stainless steel 304 prior to (unexposed) and after (exposed) exposure in artificial sweat for one week according to the non-modified EN 1811 standard protocol.

Figure S2 shows changes in electrochemical activity (open circuit potential, OCP) with time investigated for massive surfaces of the least (grade 303) and the most (grade 316L (d)) corrosion-resistant austenitic stainless steel grades,, 303 and 316L (d), as these grades showed large differences in Ni release (Figs. 2 in manuscript). The figure graphs represent average findings from three coupons.is shown in average of OCP.

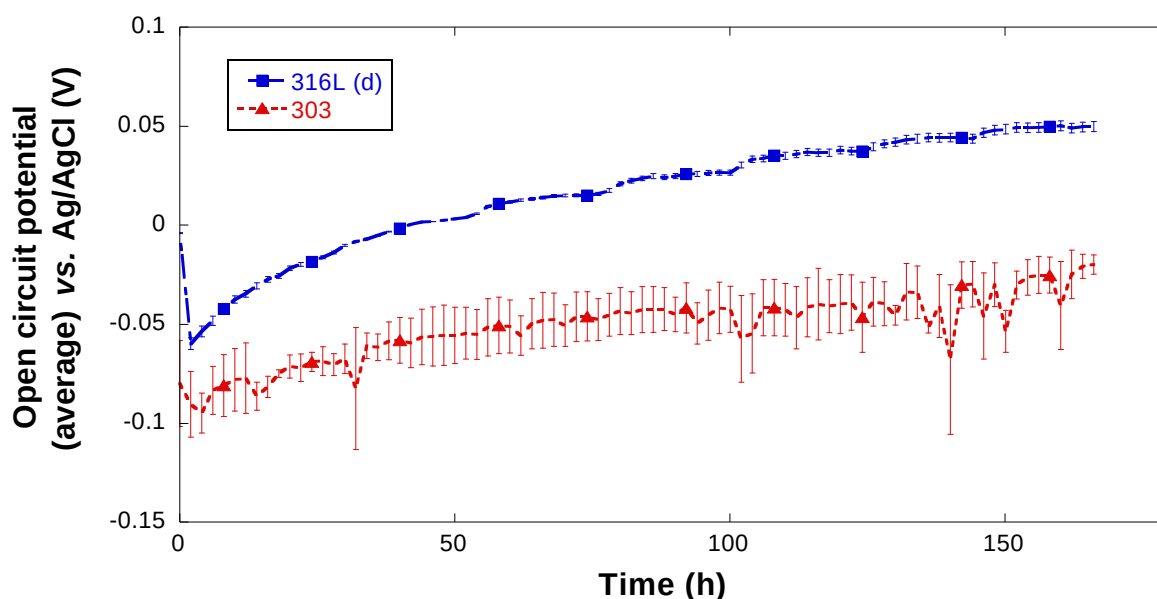


Figure S2. Changes in open-circuit potential (OCP) with time for massive surfaces of 316L (d) (blue line) and 303 (red line) exposed in artificial sweat for 168 h at room temperature. The average OCP of three samples of each grade respectively for the same exposure conditions is shown with the error bars representing the standard deviation between the triplicate samples. The OCP curve of one representative sample is shown in the main manuscript in Figure 5.

Ni and Co concentrations ($\mu\text{g/L}$) in the artificial sweat samples tested according to the EN 1811 standard or modified methods are presented in Table S1 and Table S2 together with the sample surface area (cm^2). The artificial sweat volume was adjusted to enable a coupon surface to volume ratio of $1 \text{ cm}^2/\text{mL}$. In the case of the powder samples, the volume of the artificial sweat was 0.05 L and the powder mass 5 mg.

Table S1. Released concentrations of Ni and Co (analysed by means of GF-AAS) in all artificial sweat solution samples tested for 1 week following the EN 1811 method. Information on the surface area for each coupon (massive forms) is included.

Grade	EN number	Surface area [cm^2]	Ni [$\mu\text{g/L}$]	Co [$\mu\text{g/L}$]
FeCr20Ni9	1.4420-blank1	x	2.318	1.134
	1.4420-blank2	x	1.508	0.837
	1.4420-a	4.50	5.249	20.77
	1.4420-b	4.43	3.586	1.048
	1.4420-c	4.40	3.749	0.68
FeCr21	1.4622-blank1	x	<LOD	0.071
	1.4622-blank2	x	<LOD	<LOD
	1.4622-a	4.43	0.145	<LOD
	1.4622-b	4.44	0.662	<LOD
	1.4622-c	4.46	0.028	<LOD
FeCr11	1.4003-blank1	x	5.214	1.445
	1.4003-blank2	x	3.145	1.186
	1.4003-a	8.14	74.02	6.128
	1.4003-b	8.06	7.258	1.08
	1.4003-c	8.00	4.836	0.803
FeCr18	1.4509-blank1	x	7.062	1.027
	1.4509-blank2	x	4.319	0.76
	1.4509-a	8.14	4.602	1.25
	1.4509-b	8.10	3.808	0.874
	1.4509-c	8.24	3.509	0.479
FeCr23Ni3	1.4062-blank1	x	0.952	0.87

	1.4062-blank2	x	<LOD	0.847
	1.4062-a	7.96	0.685	1.001
	1.4062-b	8.14	0.633	0.889
	1.4062-c	8.14	0.851	0.973
LDX2101	1.4162-blank1	x	0.368	<LOD
	1.4162-blank2	x	0.059	<LOD
	1.4162-a	5.40	1.751	0.297
	1.4162-b	5.37	2.128	1.053
	1.4162-c	5.42	0.934	0.053
430	1.4016-blank1	x	0.417	0.217
	1.4016-blank2	x	0.479	0.258
	1.4016-a	5.70	1.475	0.478
	1.4016-b	5.69	1.036	0.359
	1.4016-c	5.66	0.742	0.082
316L (a)	1.4404-blank1	x	1.158	0.204
	1.4404-blank2	x	0.483	0.068
	1.4404-a	4.55	6.883	0.223
	1.4404-b	4.50	6.172	0.318
	1.4404-c	4.51	7.992	0.367
304	1.4301-blank1	x	0.461	0.018
	1.4301-blank2	x	0.669	<LOD
	1.4301-a	6.15	5.333	0.023
	1.4301-b	6.11	5.439	0.052
	1.4301-c	6.13	5.216	0.122
316LVM	1.4441-blank1	x	1.13	0.137
	1.4441-blank2	x	1.166	<LOD
	1.4441-a	11.8	10.35	<LOD
	1.4441-b	11.8	10.03	0.013
	1.4441-c	11.8	10.5	0.149
303	1.4305-blank1	x	1.414	0.046
	1.4305-blank2	x	0.648	<LOD
	1.4305-a	11.7	190.6	3.405
	1.4305-b	11.8	282.7	7.336

	1.4305-c	11.9	377.2	7.305
630	1.4542-blank1	x	1.045	<LOD
	1.4542-blank2	x	0.996	<LOD
	1.4542-a	11.8	10.55	0.074
	1.4542-b	11.8	9.25	0.208
	1.4542-c	11.9	111.4	3.057
316L (c)	1.4435-blank1	x	1.61	0.263
	1.4435-blank2	x	0.654	0.005
	1.4435-a	11.8	39.82	0.239
	1.4435-b	11.8	32.73	0.301
	1.4435-c	11.9	39.48	0.442
316L (b)	1.4404m.g.-blank1	x	2.819	0.296
	1.4404m.g.-blank2	x	1.427	<LOD
	1.4404m.g.-a	11.9	9.841	0.208
	1.4404m.g.-b	12.1	7.778	0.344
	1.4404m.g.-c	11.8	7.859	0.238
FeCr21Ni11	1.4472-blank1	x	1.268	<LOD
	1.4472-blank2	x	0.862	<LOD
	1.4472-a	12.0	5.373	0.274
	1.4472-b	11.8	5.715	0.279
	1.4472-c	11.9	5.471	<LOD
Alloy 605	2.4964-blank1	x	0.255	1.659
	2.4964-blank2	x	2.259	0.987
	2.4964-a	5.3	46.97	402.9
	2.4964-b	4.9	45.82	369.1
	2.4964-c	4.7	40.13	340.4
IN718	2.4668-blank1	x	1.408	0.12
	2.4668-blank2	x	3.443	0.416
	2.4668-a	4.00	143.9	1.099
	2.4668-b	4.11	287.1	2.625
	2.4668-c	4.08	309.9	1.784
CoCr28Mo6	CoCr-blank1	x	<LOD	0.546
	CoCr-blank2	x	1.154	2.741

	CoCr-a	4.58	1.877	502.1
	CoCr-b	5.31	1.082	579.5
	CoCr-c	5.13	1.519	366.5
R30008	2.4711-blank1	x	4.169	0.832
	2.4711-blank2	x	3.572	0.567
	2.4711-a	4.73	45.17	189.4
	2.4711-b	4.81	40.79	230.1
	2.4711-c	4.70	45.61	263.2

x – blank samples without any massive or powder test items; <LOD – below limit of detection

Table S2. Released concentrations of Ni and Co (analysed by means of GF-AAS) in all artificial sweat solution samples tested for 1 week following the modified EN 1811 method. Information on the surface area for each coupon (massive forms) is included.

Grade	EN number	Surface area [cm²]	Ni [µg/L]	Co [µg/L]
304 (a)	1.4301-blank	x	0.602	0.34
	1.4301-a	6.7	7.039	0.64
	1.4301-b	6.7	7.849	0.43
	1.4301-c	6.7	8.488	0.256
316L (d)	1.4404-blank	x	0.029	0.034
	1.4404-a	6.38	9.512	0.578
	1.4404-b	6.38	9.128	0.618
	1.4404-c	6.38	8.074	0.695
430 (a)	1.4016-blank	x	<LOD	<LOD
	1.4016-a	6.72	0.547	0.026
	1.4016-b	6.72	<LOD	0.128
	1.4016-c	6.72	<LOD	<LOD
LDX2101 (a)	1.4162-blank	x	<LOD	0.591
	1.4162-a	6.72	0.26	3.349
	1.4162-b	6.72	0.423	<LOD
	1.4162-c	6.72	0.157	0.156
Ni	Ni-blank	x	0.199	0.462
	Ni-a	0.424	6178	1.051
	Ni-b	0.424	4550	0.147
	Ni-c	0.424	3388	0.67
Co	Co-blank	x	<LOD	13
	Co-a	28.0	5.148	68280
	Co-b	29.2	5.088	71280
	Co-c	20.9	3.362	68460
304-p	1.4301-blank	x	<LOD	<LOD
	1.4301-a	19.2	1.392	<LOD
	1.4301-b	18.4	0.661	<LOD
	1.4301-c	18.3	0.463	<LOD
316L-p	1.4404-blank	x	<LOD	<LOD
	1.4404-a	15.9	3.366	<LOD
	1.4404-b	16.2	3.765	0.058
	1.4404-c	15.9	2.016	<LOD
430-p	1.4016-blank	x	<LOD	2.663
	1.4016-a	15.1	0.24	0.159
	1.4016-b	14.7	<LOD	<LOD
	1.4016-c	15.3	<LOD	<LOD
IN625-p	2.4856-blank	x	<LOD	<LOD
	2.4856-a	20.6	27.37	2.558
	2.4856-b	21.8	38.74	1.464

	2.4856-c	20.9	29.98	1.307
Ni-p	Ni-blank	x	35	<LOD
	Ni-a	41.3	1704	0.424
	Ni-b	41.0	1418	0.871
	Ni-c	40.1	1435	0.087
Co-p	Co-blank	x	<LOD	<LOD
	Co-a	13.9	<LOD	N/A
	Co-b	14.8	<LOD	29270
	Co-c	13.4	<LOD	28420

x – blank samples do not contain massive or powder test items; <LOD – below limit of detection; N/A – not analyzed due to too low sample volume.

Table S3 presents the bioaccessible concentration of released Ni and Co from all alloys calculated as the concentration of the released metal (normalized on metal mass) from the alloy compared with corresponding release of the same metal from the pure metal exposed at identical conditions (eq. (S1)). It also shows the relative bioaccessibility of released Ni and Co, as determined by eq. (S2).

$$\text{Bioaccessible concentration} = \frac{\text{Released concentration alloy}_{\text{Co or Ni}} \left(\frac{\mu\text{g}}{\text{g}} \right)}{\text{Released concentration pure metal}_{\text{Co or Ni}} \left(\frac{\mu\text{g}}{\text{g}} \right)} * 100\% \quad \text{eq. (S1)}$$

$$\text{Relative bioaccessibility} = \frac{\frac{\text{Released amount alloy}_{\text{Co or Ni}} \left(\frac{\mu\text{g}}{\text{g}} \right)}{\text{Bulk content alloy}_{\text{Co or Ni}} (\text{wt}\%)}}{\frac{\text{Released amount pure metal}_{\text{Co or Ni}} \left(\frac{\mu\text{g}}{\text{g}} \right)}{\text{Bulk content pure metal}_{\text{Co or Ni}} (\text{wt}\%)}} \quad \text{eq. (S2)}$$

Table S3. Bulk composition, bioaccessible concentration, and relative bioaccessibility of Ni and Co released from the different as-received massive alloys exposed to artificial sweat for one week following the EN 1811 standard method. Release data is normalized on the metal mass. Results for Ni and Co metals are included for comparison (a 100% bioaccessible concentration and a relative bioaccessibility of 1 for the pure metals per definition, Eqs. S1 and S2).

Grade	EN no.	wt.% Ni		Relative bioaccess. of Ni	wt.% Co		Relative bioaccess. of Co
		Bulk	Bioaccess. conc.		Bulk	Bioaccess. conc.	
FeCr11	1.4003	0.38	0.53	1.4	0.015	0.0094	0.63
430	1.4016	0.11	0.011	0.098	0.019	0.00060	0.029
FeCr18	1.4509	0.21	<LOD	<LOD	0.026	0.0010	0.040
FeCr21	1.4622	0.3	0.014	0.045	0.02	<LOD	<LOD
630	1.4542	4.5	0.27	0.059	0.13	0.0019	0.014
304	1.4301	9	0.13	0.014	0.16	0.00042	0.0026
303	1.4305	8.3	1.8	0.21	0.146	0.010	0.070
LDX2101	1.4162	1.6	0.020	0.013	0.039	0.0018	0.047
FeCr23Ni3	1.4062	2.6	0.0073	0.0030	0.068	0.00080	0.011
316LVM	1.4441	15	0.057	0.0040	0.048	0.000045	0.00090
FeCr20Ni9	1.442	8.5	0.081	0.010	0.27	0.063	0.23
316L (a)	1.4404	10	0.087	0.0090	0.2	0.00060	0.0032
316L (b)	1.4404 m.g.	10	0.039	0.0040	0.12	0.00020	0.0016
316L (c)	1.4435	13	0.22	0.017	0.095	0.00030	0.0034
FeCr21Ni11	1.4472	11	0.027	0.0020	0.033	0.00030	0.0092
IN718	2.4668	53	3.0	0.056	0.03	0.0051	0.17
R30008	2.4711	16	0.73	0.046	39	1.1	0.029
Alloy 605	2.4964	11	0.32	0.029	50	0.74	0.015
CoCr28Mo6	CoCr	0.21	0.0030	0.014	65	1.0	0.015
M-Ni-m	Ni	99.995	100	1	0.0001	0.0015	15
M-Co-m	Co	0.0183	0.023	1.3	99.9	100	1

<LOD – released metal concentrations below limits of detection.

The bioaccessible concentration and relative bioaccessibility of released Ni and Co from all alloy powders calculated as the concentration of the released metal (normalized on surface area and normalized on metal mass) from the alloy compared with corresponding release of the same metal from the pure metal powder exposed at identical conditions are presented in Tables S4 and S5, respectively. The bioaccessible concentration and relative bioaccessibility of released Ni and Co were determined using eq. 2 and eq. 3 (main manuscript) when normalized on surface area, while determined using eq. S1 and eq. S2 when normalized on metal mass.

Table S4. Bulk composition, bioaccessible concentration, and relative bioaccessibility of Ni and Co for the different alloy powders exposed to artificial sweat for one week following the modified EN 1811 method. Release data is normalized on the surface area. Results for Ni and Co metals are included for comparison (a 100% bioaccessible concentration and a relative bioaccessibility of 1 for the pure metals per definition, Eqs. 2 and 3).

Grade	EN number	wt-% Ni		Relative bioaccess. of Ni	wt.% Co		Relative bioaccess. of Co
		Bulk	Bioaccess. conc.		Bulk	Bioaccess. conc.	
304-p	1.4301	9.4	0.12	0.013	0.07	<LOD	<LOD
316L-p	1.4404	10.5	0.52	0.050	0.06	5.8E-05	9.6E-04
430-p	1.4016	0.11	0.015	0.13	0.03	<LOD	<LOD
IN625-p	2.4856	63.68	4.2	0.065	0.01	0.0041	0.41
Ni-p	Ni	99.999	100	1	0.0120	0.00050	0.045
Co-p	Co	0.0027	<LOD	<LOD	98.7	100	1

<LOD – released metal concentrations below limits of detection.

Table S5. Bulk composition, bioaccessible concentration, and relative bioaccessibility of Ni and Co for the different alloy powders exposed to artificial sweat for one week following the modified EN 1811 method. Release data is normalized on the metal mass. Results for Ni and Co metals are included for comparison (a 100% bioaccessible concentration and a relative bioaccessibility of 1 for the pure metals per definition, Eqs. S1 and S2).

Grade	EN number	wt% Ni		Relative bioaccess. of Co	wt% Co		Relative bioaccess. of Co
		Bulk	Bioaccess. conc.		Bulk	Bioaccess. conc.	
304-p	1.4301	9.4	0.059	0.0060	0.07	<LOD	<LOD
316L-p	1.4404	10.5	0.21	0.020	0.06	7.2E-05	1.2E-03
430-p	1.4016	0.11	0.0050	0.049	0.03	<LOD	<LOD
IN625-p	2.4856	63.68	2.1	0.033	0.01	0.0064	0.63
Ni-p	Ni	99.999	100	1	0.0120	0.0017	0.14
Co-p	Co	0.0027	<LOD	<LOD	98.7	100	1

<LOD – released metal concentrations below limits of detection.