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Bioaccessibility Studies of Ferro-Chromium Alloy Particles for a Simulated Inhalation Scenario: A Comparative Study With the Pure Metals and Stainless Steel

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Original Research

ABSTRACT

The European product safety legislation, REACH, requires that companies that manufacture, import, or use chemicals demonstrate safe use and high level of protection of their products placed on the market from a human health and environmental perspective. This process involves detailed assessment of potential hazards for various toxicity endpoints induced by the use of chemicals with a minimum use of animal testing. Such an assessment requires thorough understanding of relevant exposure scenarios including material characteristics and intrinsic properties and how, for instance, physical and chemical properties change from the manufacturing phase, throughout use, to final disposal. Temporary or permanent adverse health effects induced by particles depend either on their shape or physical characteristics, and/or on chemical interactions with the particle surface upon human exposure. Potential adverse effects caused by the exposure of metal particles through the gastrointestinal system, the pulmonary system, or the skin, and their subsequent potential for particle dissolution and metal release in contact with biological media, show significant gaps of knowledge. In vitro bioaccessibility testing at conditions of relevance for different exposure scenarios, combined with the generation of a detailed understanding of intrinsic material properties and surface characteristics, are in this context a useful approach to address aspects of relevance for accurate risk and hazard assessment of chemicals, including metals and alloys and to avoid the use of in vivo testing. Alloys are essential engineering materials in all kinds of applications in society, but their potential adverse effects on human health and the environment are very seldom assessed. Alloys are treated in REACH as mixtures of their constituent elements, an approach highly inappropriate because intrinsic properties of alloys generally are totally different compared with their pure metal components. A large research effort was therefore conducted to generate quantitative bioaccessibility data for particles of ferro-chromium alloys compared with particles of the pure metals and stainless steel exposed at in vitro conditions in synthetic biological media of relevance for particle inhalation and ingestion. All results are presented combining bioaccessibility data with aspects of particle characteristics, surface composition, and barrier properties of surface oxides. Iron and chromium were the main elements released from ferro-chromium alloys upon exposure in synthetic biological media. Both elements revealed time-dependent release processes. One week exposures resulted in very small released particle fractions being less than 0.3% of the particle mass at acidic conditions and less than 0.001% in near pH-neutral media. The extent of Fe released from ferro-chromium alloy particles was significantly lower compared with particles of pure Fe, whereas Cr was released to a very low and similar extent as from particles of pure Cr and stainless steel. Low release rates are a result of a surface oxide with passive properties predominantly composed of chromium(III)-rich oxides and silica and, to a lesser extent, of iron(II,III)oxides. Neither the relative bulk alloy composition nor the surface composition can be used to predict or assess the extent of metals released in different synthetic biological media. Ferro-chromium alloys cannot be assessed from the behavior of their pure metal constituents. Integr Environ Assess Manag 2010;6:441–455. © 2009 SETAC

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INTRODUCTION

Human exposure to metal-containing particulate matter in our daily environment has become an issue of general focus in society. The exposure may, for example, relate to occupational activities, such as production of metals and further development of products, while other particles originate from combustion or traffic situations. Different kinds of particles exist with varying composition ranging from pure metals, metallic components, and metal alloys with oxidized surfaces to particles consisting of metal oxides or compounds. Potential adverse effects on human health caused by exposure to metal particles or metal compounds through the main routes, that is oral (ingestion), dermal (skin contact), and/or inhalation, and their subsequent potential for particle dissolution and metal release in contact with the human body, show significant gaps of knowledge.

Product safety legislation, including the REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) regulation currently being enacted in the European Union, requires that industry demonstrates that any human or environmental risks associated with the manufacture and uses of products placed on the market are identified and adequately controlled throughout their life cycle. This process

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involves a detailed assessment of the hazards of the chemical substance or material in question against a series of health and environmental effects endpoints by generating and interpreting data which can be used to determine the hazard classification and to combine the effects data with exposure data to identify risks to humans and the environment as the basis for formulating risk reduction strategies. It is essential that such important decisions are based on the realistic interpretation of high-quality scientific data taking into account the emphasis on use of alternatives to animal testing.

Alloys represent the most significant and widespread use of many metals, and therefore, it is important to develop a detailed understanding of the characteristics and behavior of this group of materials in which the operational characteristics typically differ from those of the constituent elements. Many alloys have existed for a very long time and the benefits of the alloy properties, such as mechanical strength, hardness, and ductility, are generally well known and documented. However, potential adverse effects induced on human health and the environment, caused upon manufacture and use of alloys in, for example, food processing and health care, are very seldom assessed and none of the alloys available and relatively few of their component elements appear to have undergone the detailed assessment required. As a result, meeting the demands of REACH suggests for many metals and alloys the generation of a large amount of new and unique data to accomplish an accurate risk assessment dossier. It is, therefore, crucial that these efforts are based on a scientifically sound approach that ensures accurate and quantitative research efforts of relevance covering all, or at least most, aspects of the potential adverse effects on human health and environment with a minimum dependence on in vivo testing.

One key parameter within this approach is to generate bioaccessibility data, in this context, the pool of released metals from a metal or alloy that potentially can be available for absorption by an organism. The metal-release process is governed by a combination of corrosion (electrochemical) and dissolution (chemical) processes, and its extent depends on a large number of interacting parameters, including barrier properties and composition of surface oxides, and prevailing environmental and exposure conditions. Scientific efforts have lately been undertaken to investigate the metal release process and to generate quantitative bioaccessibility data for Fe and Cr-containing metals as massive sheet (Leygraf et al. 1979; Hultquist et al. 1984; Chiba et al. 1997; Flint et al. 1998; Herting et al. 2005, 2007, 2008a, 2008b, 2008c, 2009; Odnevall Wallinder et al. 2006; Summer et al. 2007). However, few studies exist to assess bioaccessibility data for Fe and Cr-containing particles (Midander et al. 2007, 2006; Ullmann 2009). To assess whether any systemic or organ toxicity in humans will occur upon exposure of such particles by means of skin contact, by means of inhalation, or by means of the gastrointestinal tract to the stomach, the generation of reliable quantitative bioaccessibility data in different synthetic biological media and its relation to surface properties and particle interactions is essential. Such assessments require, in addition, information on the chemical form of released metals and the complexing capacity of the surrounding medium, parameters governing the bioavailability (the fraction of released metal which has the chemical form to be readily absorbed to humans through different routes).

Under the United Nations Globally Harmonized System of Classification and Labeling of Chemicals (UN 2003), alloys are explicitly addressed as simple mixtures of metals, with hazard identification and classification based on the intrinsic properties of the individual metal constituents from which they are derived. This simplified view is, in the case of an alloy such as stainless steel, highly erroneous. From a metal-release perspective, this is evident when comparing released amounts of Cr, Fe, and Ni from stainless steel grade AISI 316L with those from the respective pure metals in different kind of media (Herting et al. 2005, 2008c). These studies indicate that stainless steel grade 316L, containing 17.2 wt% Cr and 10.7 wt% Ni actually behaves as a Cr-based material containing 0.02 wt% Fe and 0.005wt% Ni, both well below the lowest threshold concentration for hazard classification (Herting 2008).

REACH recognizes alloys as special preparations: *''Alloys are preparations under REACH albeit special ones where the properties of the preparation do not always simply match the properties of the components''; ''When assessing the risk of the use of 1 or more substances incorporated into a special preparation (for instance alloys), the way the constituent substances are bonded into the chemical matrix should be taken into account.''* (EC Directive 67/548/EEC, Annex I).

Several of the common alloying elements, including Al, Cr, Fe, Mn, Ni, and Ti, do not occur naturally in their metallic state but in oxidized forms in minerals and ores. Significant energy is required to convert these ores into the corresponding metal or alloy, normally by means of carbothermic or electrolytic processes. The surface of these metals and alloys containing them will undergo spontaneous oxidation on exposure to air, but it is not possible to predict the composition of the surface layer. For alloys, it is highly likely that it is the chemistry of the alloy system and not its metallurgy that is the most important, if not the only, factor which determines behavior when exposed to biological systems. The nature and stability of the surface oxide are important, because it prevents direct contact between the surrounding medium and the nonoxidized alloy and it regulates the bioaccessibility, defined as the degree to which a substance is soluble under physiological conditions and, therefore, potentially available for absorption into systemic circulation, of the ions of the component metals. It is very likely that for any alloy system there is a concentration of the dominant element below which its influence over the characteristics of the alloy deteriorates. For example, the limit for Cr in Cr-containing ferroalloys such as stainless steel appears to be 10.5–13 wt% (Chandler and Hudson 1994).

It is reasonable to expect that the surface composition will determine acute, or short-term toxicity characteristics; the surface composition, together with amount, rate, and chemical speciation of released metals of the component metals, will determine local long term effects; and that metal release rates will determine systemic effects when toxicokinetics and metabolism are taken into account.

Although the above approach can be used to demonstrate the way the alloy components are *''bonded into the chemical matrix,''* it cannot on its own determine the degree to which the dominant element occupies the surface, changes in surface composition as a result of exposure to a biological system, or the rate at which metal component elements are released from or through the surface oxide into the surrounding medium. Clearly, solubilities of the components of the surface oxide are important indicators to what is likely to happen.

Development of a detailed picture of an alloy by means of the in vitro processes which measure the behavior of an alloy exposed to synthetic biological media relevant to the different routes of exposure effectively provides a fingerprint of the material under test and can be used as the basis for informed decisions by toxicologists to 1) justify use of read across from effects data for similar materials, 2) group similar materials with respect to health and environmental effects, 3) demonstrate the differences in bioaccessibility between metals on their own and when included in an alloy, and 4) make scientifically sound proposals for hazard classification and risk assessment.

The risk assessment of ferro-chromium alloys, using data generated within this study as proposed above, is performed by the Finnish Institute of Occupational Health as a part of a large research effort investigating ferro-chromium particles with respect to human health and environmental endpoints (Stockmann-Juvala et al. 2009). A joint publication covering health aspects of human exposure to ferro-chromium alloy particles, simulated in vitro by means of bioaccessibility testing, will be published when the full risk assessment is finalized.

The aim of this study is to summarize the bioaccessibility data generated for the main alloy constituents of particles of ferro-chromium alloys exposed to different synthetic biological media in vitro. The extent of metal release is discussed in relation to particle and surface characteristics and composition, also taking into account aspects of chemical speciation of released metals. For comparison, data generated with a similar approach has been conducted on particles of pure Fe, pure Cr, and stainless steel grade AISI 316L. Generated results form a unique set of data that aim to improve the current state of knowledge of dissolution properties and metal release of ferro-chromium particles.

MATERIALS AND METHODS

Test materials

A typical high carbon ferro-chromium alloy, in the following denoted FeCr, used as a master alloy for further production of more advanced alloys, primarily stainless steels, was supplied and prepared by Vargön Alloys. This alloy is produced by a reduction process of Cr ore (chromite). Phases of the microstructure are primarily chromium ferrite and primary carbides (Lesko and Navara 1996). The raw materials used for the production were originally from Turkey, Albania, and Madagascar. English and Colombian coke was used as the reduction agent, and small amounts of quartz, bauxite, and alumina-rich slag were added to adjust the slag composition. The FeCr alloy was cast in layers and transferred to a cooling bed. After 2 d, when the alloy reached ambient temperature, the alloy was crushed and sieved. A sample of 1 kg was taken from the 25–75 mm commercial fraction and further crushed in a laboratory jaw crusher (Swedala TRF 2513R), ground in a ring mill for 4 min (Siebtechnic with grinding barrel of chromium steel), and finally sieved again. A relatively fine fraction with particles less than 63 μm in diameter was used for the bioaccessibility investigation.

For comparison, another type of a commercially available FeCr alloy used as a master alloy for stainless steel production was supplied by Outokumpu (chargechrome). This alloy, denoted FeCr', was prepared in a similar way as for FeCr. Further comparison was made with particles of pure Fe (99.96 wt%), pure Cr (99.76 wt%), and stainless steel (AISI 316L). Detailed information on particle and surface characteristics of these particles are given elsewhere (Ullmann 2009). Chemical bulk compositions of particles of the FeCr alloys and stainless steel are given in Table 1 based on information supplied by the manufacturers.

Particle and surface characterization

The specific surface area (m^2/g) per weight was measured by means of BET-analysis (adsorption of N at cryogenic condition) using a Micromeritics Gemini V instrument. Measurements of N adsorption were performed at 5 different partial pressures at 5 different local points (standard BET method) with a standard deviation between these replicas of less than 1%.

Particle size distribution in solution (PBS, pH 7.2) was measured by laser diffraction using Malvern Mastersizer 2000 equipment with a Hydro SM dispersion unit. Refractive indexes of 2.81, and water, 1.33 (because it was the solvent of the test media), were used as input parameters for the size measurements and standard operational conditions were applied. Triplicate samples were tested for each material to provide a valid statistical result.

Particle morphology was investigated by means of scanning electron microscopy (SEM), using a PHILIPS XL30ESEM instrument.

Surface compositional analysis was performed using X-ray photoelectron spectroscopy, XPS. Spectra were recorded using a Kratos AXIS UltraDLD X-ray photoelectron spectrometer (Kratos Analytical) using a monochromatic Al X-ray source (150 W) on areas approximately sized 700 × 300 μm. Wide spectra were run to detect elements present in the outermost surface oxide (information depth of a few nanometers) at 5 different locations. Detailed high resolution spectra (20 eV pass energy) were acquired for the main compositional elements of each test item including carbon (C1s). No sputtering was made, because this may alter the chemical state of surface components. The test items were fixed on copper tape to avoid any contamination of the instrument with airborne particles. All binding energies were calibrated by assigning the carbon hydrocarbon peak (C–H, or C–C) to 285 eV. All peak areas were determined by assigning a linear base line.

Table 1. Chemical bulk composition of the 2 different FeCr materials and 316L stainless steel in weight%

	Fe	Cr	Co	Cu	Mn	Mo	Ni	Si	Ti	V	Zn	C
FeCr	25	67	0.04	0.007	0.17	<0.004	0.4	1.1	0.08	0.13	<0.005	7.1
FeCr'	32	54	0.04	–	–	<0.001	0.2	5.1	–	0.14	–	6.9
316L	65.5	18.5	–	–	1.4	2.3	11.6	0.65	–	–	–	0.05

Synthetic biological test media

In vitro immersion tests were carried out in 4 different synthetic biological media of a pH ranging from 1.5 to 7.4. Artificial gastric fluid (GST, pH 1.5) mimics the very harsh digestion milieu of high acidity in the stomach (Hamel et al. 1998; ASTM 2003). Artificial lysosomal fluid (ALF, pH 4.5) is thought to simulate intracellular conditions in lung cells following phagocytosis and represents relatively harsh conditions (Stopford et al. 2003). Gamble's solution (GMB, pH 7.4) mimics interstitial fluid within the deep lung under normal health conditions (Stopford et al. 2003). Phosphate buffered saline (PBS, pH 7.4), is a standard physiological solution that mimics the ion strength of human blood serum. It is widely used in the research (Norlin et al. 2002) and medical health care community (Hanawa 2004; Okazaki and Gotoh 2005) as a reference test solution for comparison of data under simulated physiological conditions. The composition and pH of the 4 different test media are given in Table 2.

The test media were selected to simulate an inhalation scenario where the particles enter the body through inhalation, and/or subsequently become ingested and translocated to the gastrointestinal tract. It should be stressed that the different test media only simulate physiological conditions to a certain extent, for example, citrate in Gamble's solution replaces proteins and acetate is added to represent organic acids (Kuhn and Rae 1988; Moss 1979; Stopford et al. 2003), as the complexity and function of the real body fluids are difficult to simulate. On the other hand, in vitro results obtained by using these simulated biological media can, in a simple way, provide information that could be relevant for a real exposure situation.

The test media were prepared using ultrapure water (18.2 M Ω cm^{-1}) and chemicals of analytical grade. All test vessels were acid-cleaned (10% HNO_3 for at least 24 h, rinsed 4 times with ultrapure water and dried in ambient air in the laboratory) to avoid any risk of contamination. The pH of ALF and GMB was adjusted using 50% NaOH (1.7 mL/L) and 25% HCl (260 μL/L), respectively. The pH of PBS was adjusted with 50% NaOH (370 μL/L). Artificial gastric fluid, pH 1.5, was prepared according to the ASTM standard using 4 g of 25% HCl solution (3.6 mL/L) diluted with ultrapure water to 1 L (ASTM 2003).

Experimental procedure

Triplicate samples containing 5 ± 0.5 mg of particles were weighed on a Mettler AT20 balance with readability of 2 μg, and placed in Nalge® polymethylpentene (PMP) jars for immersion in the different test media. In vitro immersion tests were conducted using a particle loading of 0.1 g/L (corresponding to 5 mg of particles per 50 mL of test media), because this amount of particles was considered to have some relevance for human exposure to particles by means of the inhalation route. The particle loading selected also enables comparison with release data generated by means of the

Table 2. Chemical composition (g/L) and pH of synthetic biological fluids used for in vitro bioaccessibility studies

Chemicals	GST pH 1.5	ALF pH 4.5	GMB pH 7.2	PBS pH 7.4
$MgCl_2$	–	0.050	0.095	–
NaCl	–	3.21	6.019	8.77
KCl	–	–	0.298	–
Na_2HPO_4	–	0.071	0.126	1.28
Na_2SO_4	–	0.039	0.063	–
$CaCl_2 \cdot 2H_2O$	–	0.128	0.368	–
$C_2H_3O_2Na$	–	–	0.574	–
$NaHCO_3$	–	–	2.604	–
$C_6H_5Na_3O_7 \cdot 2H_2O$	–	0.077	0.097	–
KH_2PO_4	–	–	–	1.36
NaOH	–	6.00	–	–
$C_6H_8O_7$	–	20.8	–	–
H_2NCH_2COOH	–	0.059	–	–
$(NH_2)_2CO$	–	–	–	–
$CH_3CHOHCO_2H$	–	–	–	–
$C_4H_4O_6Na_2 \cdot 2H_2O$	–	0.090	–	–
$C_3H_5NaO_3$	–	0.085	–	–
$C_3H_3O_3Na$	–	0.086	–	–
HCl 25%	4	–	–	–

OECD Transformation/Dissolution Protocol as well as previous studies on bioaccessibility of particles (UN 2005; Midander et al. 2006). The particle loading can always be discussed in the context of its relevance, and the reasoning about this is not straightforward. The real deposition of particles in the human lung when inhaled depends on several factors such as particle concentration in the air, size of particles, and respiratory intensity. Also the site of deposition depends on these factors and at specific ''hot spots'' the total amount of particles deposited may locally become relatively high (Martonen et al. 1992a,b; Miki et al. 1992; Balásházy et al. 2000).

A total of 50 mL of test media were added to the particles in the test vessels before exposure during various time periods in a Stuart platform rocker S180 incubator, regulated at a temperature of $37 \pm 0.5\,^{\circ}C$, with bilinear shaking of 25 cycles per min. After immersion periods of 2, 4, 8, 24, and 168 h, the pH of samples was measured to confirm that no unusual degradation of the test media had occurred. Also a longer immersion time period of 24 d in total was investigated in the ALF test media. One blank reference containing only the test media without any particles was incubated together with the immersion of triplicate samples for each immersion period.

After the exposure, the particles were separated from the test media by centrifugation for 10 min at 704 relative centrifugal force (rcf). Fine particles that remained floating on the solution surface after centrifugation were removed using Pasteur pipettes. The resulting supernatant solution was decanted into low density polyethylene (LDPE) storage flasks and acidified with 65% HNO_3 to a pH less than 2 before any metal analysis.

All vessels and equipment used to accomplish the experimental procedures described were acid cleaned in 10% HNO_3 for 24 h, rinsed with ultrapure water for 4 times and dried in ambient air in the laboratory to avoid any risk of contamination.

Metal analysis

Except for detailed and kinetic information on iron and chromium release rates, a screening investigation was conducted to assess potential release of other metals from the FeCr particles. This screening included analysis of Al, Cu, Mg, Mn, Mo, Ni, Si, Ti, and V and was performed by means of inductively coupled plasma optical emission spectroscopy, ICP-OES. Screening was performed for selected samples exposed in GST (Perkin Elmer Optima 2000 DV) and in a second stage also for samples exposed for 24 h in ALF, GMB, and PBS (Varian Vista Ax Pro). Detailed analysis of Ni was conducted for samples exposed for 24 and 168 h in GST and ALF by means of graphite furnace atomic absorption spectroscopy, GF-AAS (Perkin Elmer AAnalyst 800). This technique enables Ni to be analyzed with a lower limit of detection (0.1 μg/L in GST and 1.5 μg/L in ALF) compared with ICP-OES (2.5 μg/L in GST).

Released concentrations of total Fe and total Cr were analyzed by means of GF-AAS. Measured results are based on 3 replicate readings of each sample and quality control samples of known concentration were analyzed consecutively. All results presented are in the following presented with the metal concentrations of blank reference samples subtracted. The limits of detection for Fe and Cr were 1 μg/L and 0.5 μg/L, respectively. If using ICP-OES for analysis, these limits of detection would have been 10 μgFe/L and 2.5 μgCr/L.

In the following, metal release rates, in terms of μg metal released per unit area and immersion period ($\mu g/cm^2\,h^{-1}$), were calculated by multiplying the measured released concentration of metal, c (μg/L), with the solution volume of the samples, V (L), the amount divided with the BET specific surface area of particles, A (m^2/g), and the time of exposure, t (h) ($=(c\times V)/(A\times 10000\times t)$). Results are also represented as a ratio (μg/μg) of the total amounts of released metal from the particles (μg), obtained by multiplying the concentration of metal (μg/L) with the solution volume of the samples (L), divided by the amount of particles loaded in the sample (μg). This ratio enables a comparison of momentary total released amounts of iron and chromium from FeCr particles and is an indicative measure of the percentage of the particles (when multiplied by a factor of 100) that has been dissolved upon immersion. Moreover, the ratio enables comparison of amounts of released metals from different particles of varying physical properties like shape, surface area, and material composition.

Chemical speciation measurements

The chemical speciation of released Cr was assessed by means of differential pulse adsorptive cathodic stripping voltammetry, DPAdCSV (Metrohm 797 VA Computrace). This technique allows determination of active (free) chromium(VI) ions compared with the total chromium concentration with its difference in concentration interpreted as the concentration of active and inactive chromium(III) ions. Measurements were conducted for FeCr particles, pure Cr particles, and 316L stainless steel particles, after 24 h of immersion in GST, ALF, PBS, and GMB. All test solutions were after exposure immediately frozen prior to analysis to ensure accurate measurements. Measurements and sample preparations were conducted according to the Metrohm application bulletin (Metrohm 2009). The DPAdCSV technique enables even lower limits of detection of chromium than GF-AAS. The limit of determination was 0.04 μg/L in GST and ALF (0.1 μg/L for GMB and PBS) and the limit of detection (detected but not determined) approximately 0.01 μg/L. For the analysis, a supporting electrolyte (2.5 mL to 10 mL of test solution) containing 0.2 mol/L sodium acetate, 0.05 mol/L diethylenetriaminepentaacetate (DTPA), and 2.5 mol/L sodium nitrate was used. Calibration was carried out individually for each sample by standard addition of chromium(VI). Analysis was performed with a prepurging time of 300 s at the hanging mercury drop electrode, followed by deposition (60 s, −1.0 V) in the differential pulse mode (pulse amplitude 50 mV) starting and ending at −1.0 V and −1.45 V, respectively (sweep rate 33.3 mV/s). The pH was adjusted to pH 6.2 ± 0.1 by using 30% NaOH.

Before analysis of active chromium(VI), the test samples were allowed to react with the complexing agent DTPA for at least 30 min to avoid any detection of chromium(III) in solution. For total Cr analysis, Cr in the test solution samples was oxidized to chromium(VI) by UV irradiation (Metrohm 705 UV Digester) using H_2O_2 (50 μL per 10 mL test solution) for 30 min at 90 °C at a pH between 4 and 6.

To ensure accurate measurements and as low limits of detection as possible for the different test media, adjustments of the test methodology were necessary. For test samples

immersed in PBS, the sensitivity decreased significantly after UV irradiation why the volume of supporting electrolyte and the deposition time were doubled. GMB is a strong buffering solution. For these test samples, an additional buffer (1 mol/L ammonia (25%) and 2 mol/L acetic acid) was added to the sample (including supporting electrolyte) to stabilize the pH. This, in turn, reduced the sensitivity which was increased by a longer deposition time (120 s). In ALF, chromium in the test solutions was complexed due to a high content of organic species, consequently lowering the sensitivity. These samples (5 mL) were, therefore, irradiated for 3 h at 90 °C together with ultrapure water (2 mL) and hydrogen peroxide (6 mL), gradually added (2 mL every h) until the samples were colorless, odorless, and without any foam.

RESULTS AND DISCUSSION

Particle size and morphology

Results of multianalytical characterization of size and morphology of the FeCr particles are presented in Figure 1.

The particle size distribution for FeCr is presented according to their mass (volume%) when dispersed in a PBS solution at nonsonicated conditions. According to the measurements, particles with sizes ranging from approximately 1 μm up to slightly more than 100 μm were present in solution. The size distribution in solution was obviously relatively broad, with a tail toward smaller particle sizes, indicating that there could be a large fraction of smaller sized particles. However, these particles did not contribute significantly to the total particle mass, or volume. According to the measurements, the volume (mass) weighted mean particle size was 27 μm. When evaluating the same size distribution data in numbers (more relevant for sub-μm and nm-sized particles) the presence of many small particles sized less than a few μm were present. These calculations resulted in a size distribution where 90% of the particles had a diameter of 3.7 μm or less. Particles of this size is relevant when considering a realistic inhalation scenario (particles with a diameter <4 μm are often referred to as inhalable) (ISO 1995). The size distribution measurements were consistent with morphology observations as depicted by the inset SEM micrograph. The image clearly shows that the FeCr alloy primarily consists of smaller-sized particles (less than 10 μm) and significantly larger particles with an estimated size varying between 10 and 50 μm. Generally, the particles have a rough shape, which is normal for mechanically produced particles. The measured BET specific surface area, 0.19 m^2/g, also agrees with the general view, with a major fraction of smaller sized particles and the presence of a few discrete and significantly larger particles. The theoretical particle diameter, calculated from the measured specific surface area (using an approximate density for FeCr of 7.0 g/cm^3 and the assumption that all particles are ideal spheres without pores) was 4.5 μm.

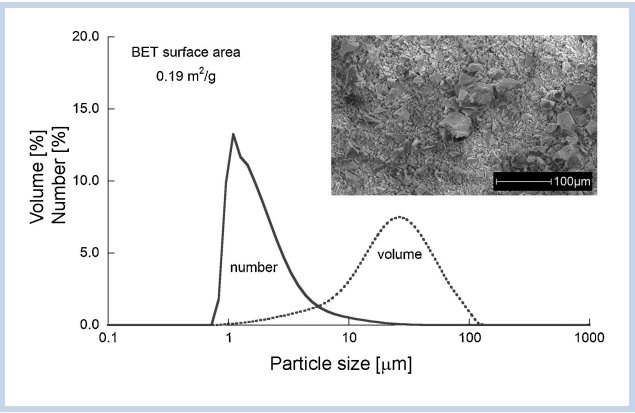


Figure 1. Particle size distribution of FeCr particles in phosphate buffered saline (PBS) solution. The inset scanning electron microscopy image shows the morphology of particles. The BET-specific surface area was determined as 0.19 m^2/g.

Bulk and surface composition

As expected, compositional variations between discrete particles were observed with generally larger particles Fe in iron with minor amounts of Si, an element primarily observed in smaller sized particles. Chromium was present to a large extent in all particles. Energy dispersive spectroscopy, EDS, measurements revealed Cr to be the main component with a Cr/(Fe+Si+Cr) ratio of 0.80. This ratio is slightly higher compared with bulk data (Table 1), giving a ratio of 0.72, a result of compositional variations between discrete particles.

The alloy surface is naturally oxidized at ambient conditions, which means that metal oxides of varying composition, thickness, and barrier properties form on the surface of the FeCr alloy. The composition and chemical state of metals in these thin surface oxides (usually nm thick) were characterized by means of X-ray photoelectron spectroscopy. Binding energies originating from both iron and chromium in their metallic states (Fe: 707.3 ± 0.04 eV; Cr: 574.3 ± 0.04 eV), and oxidized states (Fe: 711.1 ± 0.7 eV; Cr: 576.6 ± 0.18 eV) were determined in agreement to literature findings (Chastain and King 1995). No unambiguous phase determination of iron and chromium oxides could be made, although it is clear that chromium was present as trivalent chromium. The peak assigned to oxidized iron was wide enough to include compounds of both 2- and 3-valent iron species. A binding energy of silicon of 101.9 ± 0.3 eV indicate silica (SiO_2) to be the main surface component (Teixeira et al. 2005). Observations of the metallic component suggest a thin surface oxide. The presence of both metallic and oxidized components suggested nm-thick surface oxides.

Multiarea compositional analysis of the surface oxide on the FeCr particles clearly revealed that the relative surface content of Cr, Fe, and Si was different from their bulk alloy relation, Figure 2. The results clearly illustrate a surface enrichment of iron (Fe/(Fe+Cr+Si)-29%) present as Fe(II) and Fe(III) oxides compared with its bulk content (24%), while the surface content of Cr (Cr/(Cr+Fe+Si)-64%) present as Cr(III)oxides was less compared with its relative bulk content (75%). A higher relative content of Si (Si/(Cr+Fe+S_i)-7%), as silica (SiO_2) was present on the surface compared with its relative low bulk alloy content (1%). This may be the result of aging and particle preparation, a phenomenon observed for dust particles of the same materials.

Metal release screening study

A screening investigation of elements released from the FeCr alloy was performed for selected samples exposed in GST and in a second stage also for samples exposed in ALF, GMB, and PBS. The purpose of the screening investigation

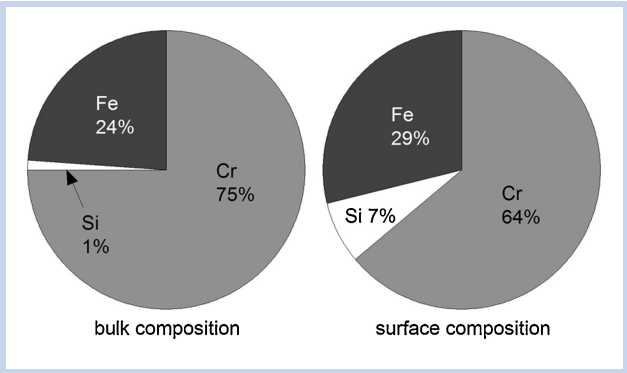


Figure 2. Relative composition (weight%) of chromium, iron and silicon in the bulk alloy (left) compared with the surface oxide determined by means of x-ray photoelectron spectroscopy for FeCr alloy particles.

was to assess if elements, other than the main constituents Fe and Cr were released in the synthetic media investigated. The results, in terms of measured metal concentrations, are compiled in Table 3. The limits of detection are generally higher in GST than for the other media investigated as a result of the use of another instrument. More details are given in the experimental section.

For most elements (Al, Cu, Mo, Ni, Ti, and V) and media, released concentrations were below or close to the limit of detection. A high concentration of magnesium was determined for the pH-neutral test medium GMB after 24 h exposure despite background subtraction of the Mg content of the test medium itself. Its presence in solution is not a pH effect because very low concentrations of Mg were determined in PBS, a medium of similar pH but very different composition compared with GMB. Magnesium is not a minor constituent of the FeCr alloy; its origin in solution likely is associated with particles of slag material from the production.

A supplier or manufacturer of an article (in this case, the FeCr alloy) which contains a hazardous substance (in this case, a metal component) at or above its classification threshold value must, according to Article 33 in the REACH regulation (EC Regulation No 1907/2006), provide sufficient information to allow safe use of the article. Except for Fe and Cr, minor elements (above 0.1 wt%) of Mn (0.17 wt%), V (0.13 wt%), Ni (0.4 wt%), and Si (1.1 wt%) were present in the FeCr alloy. The screening study revealed Mn in concentrations ranging from 4 to 10 μg/L in ALF, GMB, and PBS after 24 h. No release of V was observed in any of the test media investigated. Silicon was released into the different media at concentrations between 70 and 120 μg/L. Previous investigations have shown more pH-neutral conditions to dissolve more silicon compared with acidic conditions (Lide 1999). This dependence was not observed in this study, which may reflect that Si was not primarily released from the alloy, but rather originates from silica slag remnants. Another reason may be related to the fact that a larger portion of the surface became enriched in silica upon exposure at the acidic conditions of ALF compared with the more pH-neutral media of PBS and GMB (to be discussed later on).

In addition to the screening investigation performed by means of ICP-OES (limit of detection 2.5 μg/L), more accurate measurements of released Ni were performed for FeCr alloy particles immersed in the acidic media ALF (pH 4.5) and GST (pH 1.5) by using GF-AAS (limit of detection 0.2 μg/L). These investigations were conducted in view of the fact that Ni compounds are placed on the candidate list of substances of very high concern by the International Chemical Secretariat (ChemSec 2009). In GST, the most aggressive test media (pH 1.5), nickel was present at a concentration of 5 μg/L for FeCr alloy particles after 24 h and 168 h. This concentration corresponds to average Ni release rates of 0.2 $\mu g/cm^2$ $week^{-1}$ (extrapolated from the concentration measured after 24 h of exposure), and 0.03 $\mu g/cm^2$ $week^{-1}$ determined after 168 h (1 week) of exposure. In ALF, the medium mimicking inflammatory conditions of the lung compartment, released concentrations of Ni from the FeCr alloy were even lower (<0.03 $\mu g/cm^2$ $week^{-1}$) and similar to background concentrations of the blank solution (no particles added) after both 24 and 168 h, respectively.

Determined average release rates of nickel in GST and ALF were considerably lower compared with the threshold value of nickel of 0.5 $\mu g/cm^2$ $week^{-1}$ in artificial sweat (CEN 1998). However, this threshold rate is relevant for skin sensitization and not for inhalation or ingestion.

Iron and chromium release rates

Detailed kinetic information was generated on the released amount of Fe and Cr from FeCr alloy particles exposed in the

Table 3. Screening of alloy elements of minor concentration in the bulk alloy released from FeCr during 24 hours of immersion in GST, ALF, GMB, or PBS[a]

	Bulk alloy composition/wt%								
Metal	**Al**	**Cu**	**Mg**	**Mn**	**Mo**	**Ni**	**Si**	**Ti**	**V**
	–	0.007	–	0.17	<0.004	0.4	1.1	0.08	0.13
	Released metal concentration/μg/L								
GST	<50	<0.7	<10	*	*	4.8	117	<10	<0.7
ALF	<0.7	<0.3	<0.1	8.6	<1.9	<2.5	<5.1	<0.1	<1.1
GMB	3.2	<0.3	2804	3.8	<1.9	<2.5	68.9	<0.1	<1.1
PBS	<0.7	1.0	2.3	9.5	<1.9	<2.5	74.9	0.8	<1.1

[a,*] = not measured; - = no information; GST = gastric fluid (pH 1.5); ALF = artificial lysosomal fluid (pH 4.5); GMB = Gamble's solution (pH 7.4); and PBS = phophosphate buffered saline (pH 7.2).

synthetic biological media investigated. The results are presented as total average metal release rates per unit surface area in Figure 3 and compiled in Table 4. Release rates of Fe and Cr are generally low, though highest in the acidic GST test media. Iron was the preferentially released element in all test media for all time periods investigated. The kinetic behavior of the release of both Fe and Cr from FeCr alloy particles was initially relatively high rates that declined toward low rates after 1 week of exposure (168 h). This behavior was representative for the exposure of particles in the most acidic test media GST and the 2 pH-neutral media GMB and PBS, respectively. However, the kinetic behavior of Fe and Cr release in ALF was slightly different, showing initially low release rates during the first hours of exposure that increased slightly, reaching a maximum before declining toward lower rates after longer exposure periods. The significance of the increasing or decreasing trend in release rates for iron and chromium can be discussed because the results for the initial time periods are based on low metal concentrations, also reflected by larger standard deviation between triplicate samples after short time periods (2, 4, and 8 h) compared with longer periods of exposure (24 and 168 h). However, all replicate samples revealed the same trend. The results are consistent with previous observations made for freshly polished stainless steel AISI 316L exposed to similar exposure conditions (Herting et al. 2006). The barrier properties and the Cr content of the surface oxide of stainless

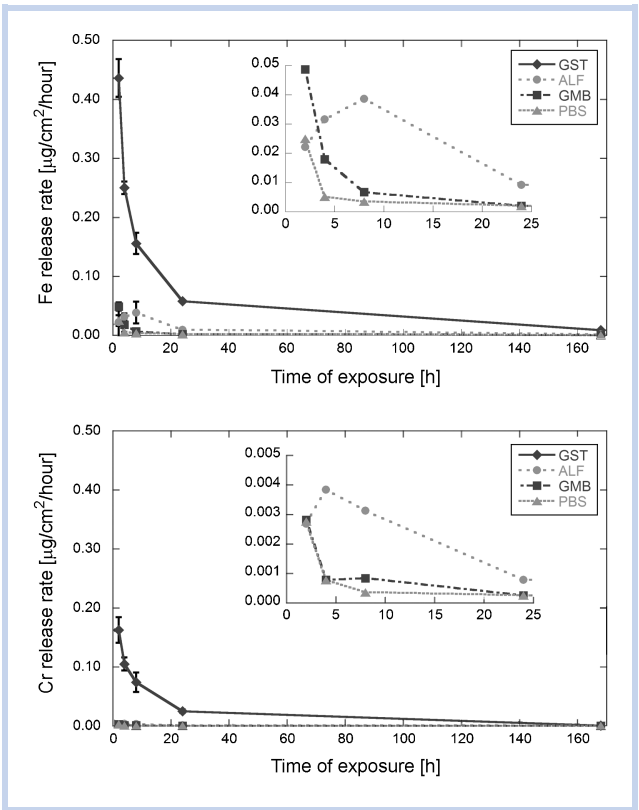


Figure 3. Release rates per unit surface area of iron and chromium from FeCr alloy particles exposed in GST, ALF, GMB, and PBS for various time periods up to 168 h (1 week). Error bars represent the standard deviation of triplicate samples. The inset graphs show in more detail initial release rates of iron and chromium in ALF, GMB and PBS. Abbreviations: GST = gastric fluid (pH 1.5); ALF = artificial lysosomal fluid (pH 4.5); GMB = Gamble's solution (pH 7.4); and PBS = phosphate buffered saline (pH 7.2).

Table 4. Release of iron and chromium from FeCr alloy particles calculated as release rates [$\mu g/cm^2\ h^{-1}$], in GST, ALF, GMB, and PBS, for all time periods investigated[a]

Element-media	GST		ALF		GMB		PBS	
Time period	Fe	Cr	Fe	Cr	Fe	Cr	Fe	Cr
2 h	0.44 ± 0.03	0.16 ± 0.02	0.022 ± 0.024	0.003 ± 0.003	0.049 ± 0.007	0.0028 ± 0.0006	0.025 ± 0.009	0.0028 ± 0.0009
4 h	0.25 ± 0.01	0.10 ± 0.01	0.032 ± 0.005	0.004 ± 0.001	0.018* ± 0.005	0.0008* ± 0.0001	0.005 ± 0.001	0.0008 ± 0.002
8 h	0.16 ± 0.02	0.07 ± 0.02	0.038 ± 0.018	0.003 ± 0.002	0.0067 ± 0.0008	0.0008 ± 0.0001	0.004 ± 0.001	0.0004 ± 0.0001
24 h	0.057 ± 0.001	0.025 ± 0.003	0.009 ± 0.001	0.0008 ± 0.0001	0.0020 ± 0.0012	0.0002 ± 0.0001	0.002 ± 0.001	0.0003 ± 0.0001
168 h	0.008 ± 0.001	0.0006 ± 0.000	0.0012 ± 0.0008	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0001 ± 0.0000	0.00002 ± 0.00001

[a,*] = result based on duplicate samples. GST = gastric fluid (pH 1.5); ALF = artificial lysosomal fluid (pH 4.5); GMB = Gamble's solution (pH 7.4); and PBS = phosphate buffered saline (pH 7.2).

steel were gradually improved with time upon exposure in ALF. It was hence anticipated that a similar process also took place for FeCr alloy particles immersed to both acidic media, ALF and GST, although at a too fast a rate in GST to be observed during the selected time periods of initial sampling. As a consequence of initial dissolution, prolonged exposure resulted in a surface oxide reconstruction and repassivation which improved its barrier properties with time.

As evident from relative surface compositional measurements by means of XPS of Cr, Fe, and Si (as oxides), the relative Cr content of the surface oxide was enhanced upon exposure in ALF, Figure 4. The lack of metallic components of the XPS spectra of Fe and Cr after 168 h suggests an increasing oxide thickness with time. The relative amount of Fe was significantly reduced compared with the unexposed surface as a result of the preferential release of Fe into solution. As a consequence, silica and chromium(III)oxides became the main surface components with iron(II, III)oxides less frequent. Differences in relative silica surface content with time are most probably related to compositional variations between particles as previously discussed.

The surface enrichment of Cr during exposure to the pH neutral media PBS (pH 7.4) was not as pronounced as observed at acidic conditions (Figure 4). This is consistent with significantly lower release rates of Fe. The relative proportion of Si (as silica) in the surface oxide was enriched compared with unexposed conditions. In contrast to most metals, silica is more soluble at pH neutral conditions compared with acidic conditions.

Total amounts of released iron and chromium

Normalization of released concentrations of metals to the BET surface area can be misleading, because the surface area usually changes with time, in particular if a large fraction of the particles is dissolved. Another way to present metal release data is as the ratio (μg/μg) between the total amounts of released Fe and Cr from the FeCr alloy particles (μg) and the amount of particles loaded (μg). This ratio is an indicative measure of the percentage of the particle mass that has been released (dissolved) into solution. To enable comparison of the extent of metal release into the different test media, average ratios are displayed in Figure 5 after 2 and 24 h in each test media and compiled for all time periods in Table 5. In all media, independent on pH, the amounts of released Fe and Cr per amount of particles loaded were very low. After 24 h, the released fraction was less than 0.4% in GST, less than 0.1% in ALF, and less than 0.05% in PBS and GST. As previously discussed, Fe was preferentially released and released to a much higher extent than Cr, despite the fact that the particle surface contains approximately 2.5 times as much Cr as Fe.

The results show that the kinetics of metal release previously discussed is not only an effect of time and dissolution and/or corrosion processes reaching relative steady state conditions. The total amounts of Fe and Cr released per amount of particles loaded increased slightly with time in the acidic test media GST and ALF, while the opposite was observed for pH-neutral GMB.

Average total amounts of Fe and Cr, released from FeCr alloy particles immersed in ALF were investigated also for a significantly longer time period than 1 week, 24 d in total. The aim of this extension was to justify any potential use of 1-week data to assess long-term effects induced by the inhalation of FeCr alloy particles. Long-term metal release data are presented in Figure 6 together with short-term released amounts of Fe and Cr per amount of particles loaded. Only a slight change in solution pH (+0.2 units) was observed over the 24-d exposure period. The results show, as

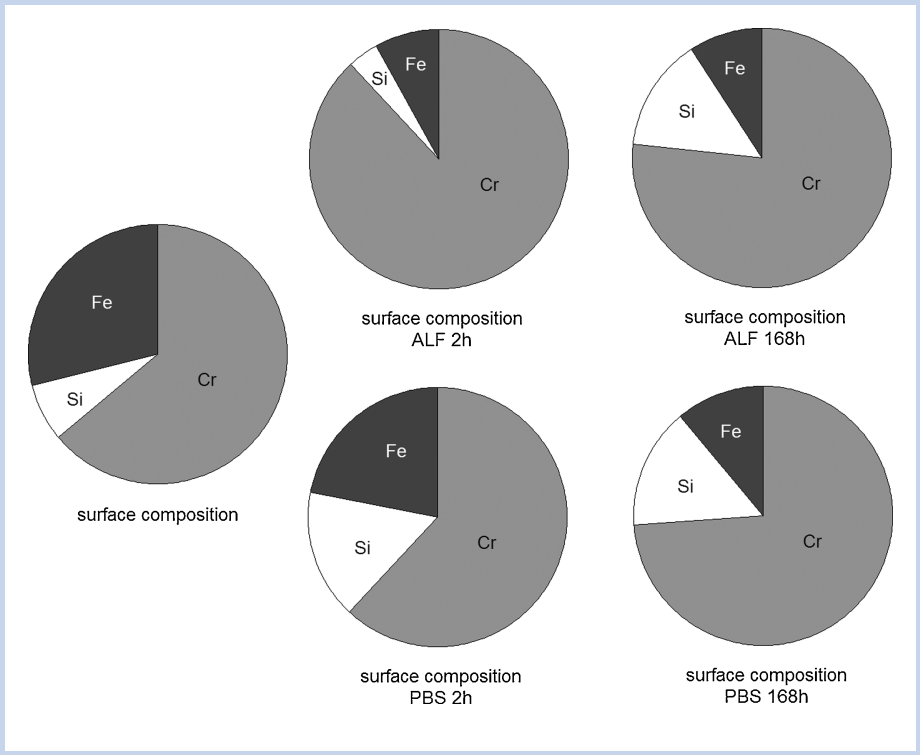


Figure 4. Changes in relative surface composition of chromium, iron and silicon (weight%) of FeCr alloy particles before and after 2 and 168 h of immersion in an acidic media, artificial lysosomal fluid (ALF; pH 4.5) and a pH-neutral media, phosphate buffered saline (PBS, pH 7.2).

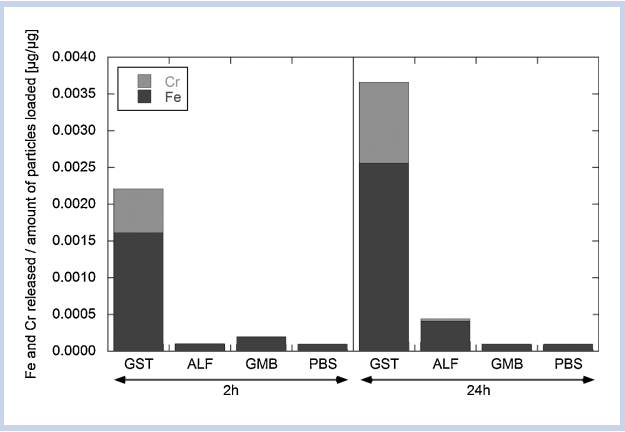


Figure 5. Total average amount of released iron and chromium from FeCr alloy particles per amount of particles loaded, in GST, ALF, GMB, and PBS after 2 and 24 h of exposure. Abbreviations: GST = gastric fluid (pH 1.5); ALF = artificial lysosomal fluid (pH 4.5); GMB = Gamble's solution (pH 7.4); and PBS = phosphate buffered saline (pH 7.2).

previously discussed, increasing amounts of Fe and Cr released during the first 8 h of exposure reaching relatively constant levels as the barrier properties of the surface oxides improve with time. Slightly lower amounts (although of low significance) with prolonged exposure time periods can be due to the fact that released metals can precipitate from solution and redeposit on the surface. For example, Fe precipitates, depending on solution composition, to $Fe(OH)_3$ or other complexes (Keddam 1995; Skeaff et al. 2008). In vitro studies in synthetic freshwater fluids have previously displayed similar behavior with decreasing Fe concentrations with time from pure Fe particles and to a smaller extent from particles of stainless steel (AISI 304) (Skeaff et al. 2008) and for the same FeCr alloy particles as used in this study (Ullmann 2009).

Released amounts of Fe and Cr were though still very low after 24 d of immersion, (less than 0.1%) and with Fe preferentially released compared with Cr. The results are comparable with 1-week data.

Comparison with stainless steel and the pure metals

In addition to the assessment of metal release from particles of the FeCr alloy, another common type of a commercially available FeCr alloy was investigated in parallel, FeCr' (BET surface area 0.32 m^2/g). This alloy revealed similar trends as observed and discussed for the FeCr alloy with very low released amounts of Fe and Cr and only a small fraction of the total amount of particles dissolved in the test media investigated (amounts of released Fe and Cr together was less than 0.2% of the amount of particles loaded). Results from the 2 FeCr alloys are presented in Figure 7, and compared with the amounts of released metal from particles of pure Fe (0.05 m^2/g) and Cr (0.04 m^2/g) as well as stainless steel grade AISI 316L (0.07 m^2/g) after 1 week of exposure in ALF. As expected, particles of the 2 FeCr alloys and stainless steel 316L released significantly less Fe compared with the pure element ($p < 0.001$ for FeCr/FeCr'/316L particles compared with pure Fe). However, more Fe was released from particles of both FeCr alloys compared with stainless steel, although still at very low levels, which is discussed later in terms of the total amount of metal released.

Table 5. Released amounts of iron and chromium from FeCr alloy particles per amount of particles loaded [µg/µg], in GST, ALF, GMB, and PBS, for all time periods investigated[a]

Element-media	GST		ALF		GMB		PBS	
Time period	Fe	Cr	Fe	Cr	Fe	Cr	Fe	Cr
2 h	0.0016 ± 0.0001	0.0006 ± 0.0001	0.0001 ± 0.0001	0.00001 ± 0.00001	0.0002 ± 0.0000	0.0000 ± 0.0000	0.0001 ± 0.000	0.00001 ± 0.00000
4 h	0.0018 ± 0.0001	0.0008 ± 0.0001	0.0002 ± 0.0000	0.00003 ± 0.00000	0.0001* ± 0.0000	0.0000* ± 0.0000	0.0000 ± 0.0000	0.00001 ± 0.00001
8 h	0.0023 ± 0.0003	0.0011 ± 0.0002	0.0006 ± 0.0003	0.00005 ± 0.00003	0.0001 ± 0.0000	0.0000 ± 0.0000	0.0001 ± 0.0000	0.00001 ± 0.00000
24 h	0.0026 ± 0.0001	0.0011 ± 0.0001	0.0004 ± 0.0000	0.00003 ± 0.00000	0.0001 ± 0.0000	0.0000 ± 0.0000	0.0001 ± 0.0000	0.00001 ± 0.00000
168 h	0.0027 ± 0.0004	0.0002 ± 0.0000	0.0004 ± 0.0003	0.0000 ± 0.0001	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.00001 ± 0.00000

[a,*] = result based on duplicate samples. GST = gastric fluid (pH 1.5); ALF = artificial lysosomal fluid (pH 4.5); GMB = Gamble's solution (pH 7.4); and PBS = phosphate buffered saline (pH 7.2).

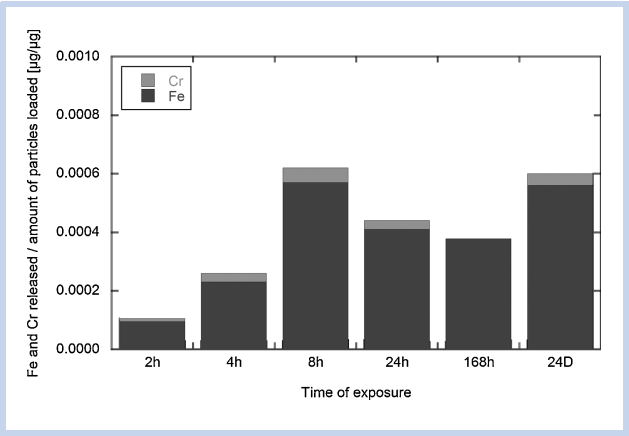


Figure 6. Total average released amounts of iron and chromium from FeCr alloy particles per amount of particles loaded in artificial lysosomal fluid (ALF, pH 4.5) during immersion periods up to 24 days (24D).

Amounts of Cr released from the FeCr alloy, 316L stainless steel, and pure Cr per particles loaded, were all significantly lower (<0.0001 µg/µg) than released amounts of Fe (<0.001 µg/µg) from the FeCr alloy and stainless steel after 1 week of exposure in ALF, Figure 7. Slightly more Cr, although still of very low amount (<0.0015 µg/µg) and comparable level with FeCr, stainless steel and pure chromium particles were released from the FeCr' alloy. Chromium released from the FeCr alloy showed large variations between triplicate samples, as evidenced from the large error bar. This reflects variations between different particle samples and the very low amounts of released Cr (corresponding to a particle dissolution of less than 0.01% of the amount of particles loaded). Similar variations were also observed for the FeCr alloy immersed in pH neutral media at concentrations less than 1 µg/L in this study (PBS and GMB), and in artificial sweat, artificial tear fluid, and the OECD transformation/dissolution test solutions of pH 6 and 8 (Hedberg et al. manuscript in preparation).

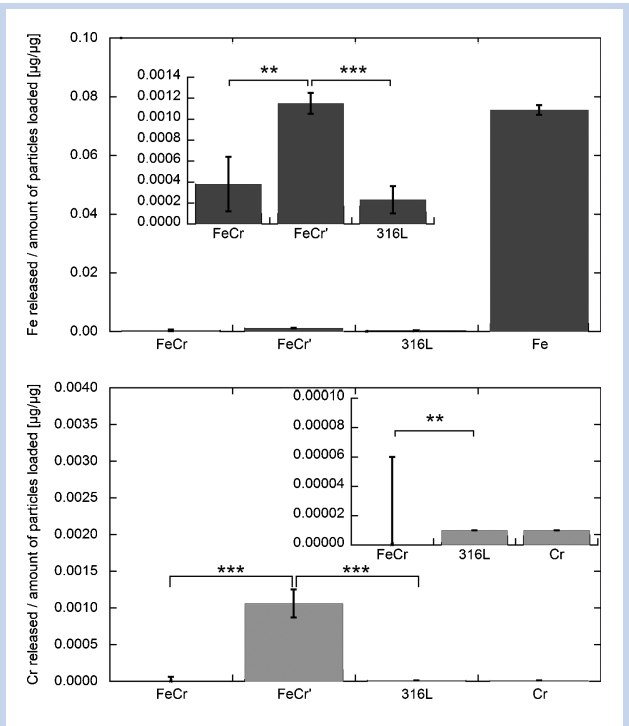


Figure 7. Comparison of released amounts of iron and chromium per amount of particles loaded from 2 different FeCr alloys, 316L stainless steel and the pure metals iron and chromium immersed for 168 h (1 week) in artificial lysosomal fluid (ALF, pH 4.5). Error bars represent the standard deviation of triplicate samples. The inset graphs show in more detail the released amounts of iron and chromium. Asterisks refer to significance levels for the test materials compared with each other; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

The total amount of metals released from the FeCr' alloy was slightly higher (corresponding to <0.3% dissolution of particles loaded) compared with the FeCr alloy (<0.1%). However, both alloys revealed a minor degree of dissolution. The main reason for the higher amounts of metals released from FeCr' particles was primarily a significantly larger specific surface area. Another reason could be a lower Cr content in the FeCr' alloy (54 wt%) compared with the FeCr alloy (67wt%), and a slightly lower relative Cr content (Cr/(Cr+Fe+Si)) in the surface oxide (56% compared with 64%), perhaps suggesting a slightly less protective surface oxide. Other particle characteristics such as particle size, specific surface area (approximately 2 times as high for FeCr' particles compared with FeCr particles), particle size distribution, and particle shape as well as differences in manufacturing processes and aging of the particles, are as important when comparing data.

The general trend in amounts of released iron and chromium holds true for both alloys (FeCr and FeCr') in all test media investigated, see Table 6. More Fe and Cr were generally released at acidic conditions (GST, ALF) compared with more pH neutral media (PBS, GMB). The release of Fe was up to 10 times higher from the FeCr alloy compared with 316L stainless steel, but more than 25 times lower compared with pure Fe exposed to identical conditions. Tables 6 and 7 summarize released amounts of Fe and Cr per amount of particles loaded and their release rates from FeCr, FeCr', 316L stainless steel, pure Fe, and pure Cr after 1 week of immersion in the test media investigated.

Comparison with fine-sized particles

Particles of the FeCr and the FeCr' alloy and of pure Fe and pure Cr, were milled and sieved through a 20-µm sieve aiming to increase the fraction of smaller sized particles for each material. This process resulted in increased BET areas, in particular pronounced for particles of the pure metals (Fe: 0.84 compared with 0.05 m^2/g, Cr: 0.91 compared with 0.04 m^2/g) but also evident for the alloys (FeCr: 0.48 compared with 0.19 m^2/g, FeCr': 0.60 compared with 0.32 m^2/g).

Figure 8 shows total released amounts of Fe and Cr in ALF after 168 h for the fine-sized materials compared with their larger-sized fractions. In general, the released amount of metals from the ''fine sized'' material was significantly higher (although still at low levels), with the exception of Cr release from FeCr' particles showing an opposite scenario. The general trend with higher amounts of released metals with decreasing size and increasing surface area is still evident when presenting data as metal release rates per unit surface area. In theory, the comparison of amounts of metal released per unit surface area should result in constant rates for the same type of particles. However, this was not the case in this

Table 6. Compilation of amounts of iron and chromium released per amount of particles loaded [μg/μg] from the FeCr and the FeCr′ alloys and particles of 316L stainless steel, pure Fe, and pure Cr immersed in GST, ALF, GMB, and PBS for 168 hours (1 week)[a]

Element-media	GST (pH 1.5)		ALF (pH 4.5)		GMB (pH 7.2)		PBS (pH 7.4)	
Material	Fe	Cr	Fe	Cr	Fe	Cr	Fe	Cr
FeCr	0.0027 ± 0.0004	0.0002 ± 0.0000	0.0004 ± 0.0003	0.0000 ± 0.0001	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000
FeCr′	0.0015 ± 0.0002	0.0011 ± 0.0001	0.0012 ± 0.0001	0.0011 ± 0.0002	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0002 ± 0.0000	0.0000 ± 0.0000
316L	0.0002 ± 0.0000	0.0000 ± 0.0000	0.0002 ± 0.0001	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000
Fe	0.073 ± 0.006	–	0.075 ± 0.002	–	0.0000 ± 0.0000	–	0.0056 ± 0.0003	–
Cr	–	0.0000 ± 0.0000	–	0.0000 ± 0.0000	–	0.0000 ± 0.0000	–	0.0000 ± 0.0000

[a]GST = gastric fluid (pH 1.5); ALF = artificial lysosomal fluid (pH 4.5); GMB = Gamble's solution (pH 7.4); and PBS = phosphate buffered saline (pH 7.2).

Table 7. Compilation of release rates of iron and chromium [μg/cm^2/hour] from the FeCr and the FeCr′ alloys and particles of 316L stainless steel, pure Fe, and pure Cr immersed in GST, ALF, GMB, and PBS for 168 hours (1 week)

Element-media	GST (pH 1.5)		ALF (pH 4.5)		GMB (pH 7.2)		PBS (pH 7.4)	
Material	Fe	Cr	Fe	Cr	Fe	Cr	Fe	Cr
FeCr	0.0086 ± 0.001	0.0006 ± 0.0001	0.0012 ± 0.0008	0.000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0001 ± 0.0000	0.0000 ± 0.0000
FeCr′	0.0028 ± 0.0003	0.0021 ± 0.0001	0.0022 ± 0.0002	0.0020 ± 0.0004	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0003 ± 0.0000	0.0001 ± 0.0000
316L	0.0021 ± 0.0001	0.0002 ± 0.0000	0.0013 ± 0.0001	0.0001 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000
Fe	0.95 ± 0.07	—	0.99 ± 0.02	—	0.0000 ± 0.0000	—	0.073 ± 0.004	—
Cr	—	0.0001 ± 0.0000	—	0.0001 ± 0.0000	—	0.0000 ± 0.0000	—	0.0000 ± 0.0000

[a]GST = gastric fluid (pH 1.5); ALF = artificial lysosomal fluid (pH 4.5); GMB = Gamblés solution (pH 7.4); and PBS = phosphate buffered saline (pH 7.2).

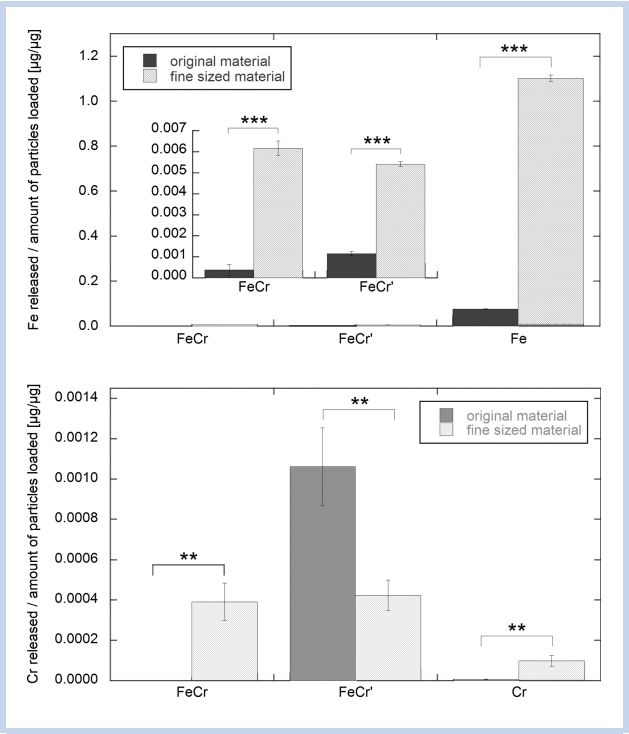


Figure 8. Comparison of released amounts of iron and chromium per amount of particles loaded from fine sized and larger sized fractions of the FeCr, and the FeCr' alloy, and particles of pure iron and chromium immersed for 168 h (1 week) in artificial lysosomal fluid (ALF; pH 4.5). Error bars represent the standard deviation of triplicate samples. The inset graph show in more detail released amounts of chromium. Asterisks refer to significance levels of release for the fine sized and original particle size of test materials compared with each other; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

study. The particle size effect influencing the extent of the metal release rate has previously been observed for stainless steel (Midander et al. 2007). Except for differences in size and surface reactivity, differences in release rates may be a result of the milling treatment of particles generating a fresh surface oxide of less protective abilities and more easily dissolved. In general, surface aging of particles and massives results in lower release rates (Herting et al. 2006; Sundberg and Jerlerud-Perez 2007; Ullmann 2009).

Chemical speciation

Bioaccessibility data generated in this study provide information about total amounts of metals released from an alloy or a pure metal, which are potentially available for humans. Any adverse effects induced by released metals can, however, not accurately be assessed without information on the bioavailability, which is determined by the chemical form of released metals (metal speciation) and whether any of these metal species can be absorbed by humans through the gastrointestinal system, the pulmonary system, or the skin. The chemical speciation of released metals is strongly correlated to the chemical composition and acidity of the test medium.

Information on the chemical speciation of released metals is seldom available, due to the complexity of the analysis and the low concentrations of released metals.

Speciation measurements of Cr were therefore conducted in this study to assess its oxidation state and its potential complexation to inorganic or organic compounds of the synthetic test media investigated. Concentrations of active chromium(VI), if any, and total Cr (the difference interpreted as active and inactive chromium(III)) released from the FeCr alloy, stainless steel AISI 316L, and pure Cr particles were determined by means of DPAdCSV after 24 h of immersion in GMB, PBS, ALF, and GST, respectively. For media of high salt content, this technique is advantageous at low Cr concentrations (<1 µg/L) compared with GF-AAS, in terms of accuracy, detection limit, and the possibility to determine speciation.

Chromium was released from the FeCr alloy, pure Cr, and stainless steel as chromium(III) species without any presence of active chromium(VI) species for all media investigated.

Measurements of released concentrations of total Cr by means of DPAdCSV were consistent and reproducible results obtained compared with total Cr concentrations measured by means of GF-AAS. All synthetic biological media except ALF showed a low capacity to form complexes with chromium as a result of their predominance of inorganic salts. ALF, which in addition contains large quantities of organic compounds showed a high capacity to form strong Cr complexes. The complexing capacity could not be defined precisely due to an interfering peak, probably deriving from the reduction of pyruvic acid to lactic acid. However, it was clearly concluded that active Cr is complexed to a high extent (almost to 100%) at low (<2 µg/L) concentrations.

CONCLUSIONS

Potential adverse effects on human health caused by the exposure of metal particles through the main routes, oral—ingestion, dermal—skin contact and/or inhalation, and their subsequent potential for particle dissolution and metal release in contact with biological media show significant gaps of knowledge. A large research effort was therefore undertaken to assess kinetic bioaccessibility data for particles of ferrochromium alloys, master alloys used for stainless steel production, upon immersion in different synthetic biological media. The main focus was placed on the generation of quantitative data of the major alloying constituents Fe and Cr, but also on the extent of release of minor elements of the alloy, including Ni, Mn, and V, metals of high concern from a REACH perspective. Bioaccessibility data is discussed in relation to particle characteristics, surface composition, and chemical speciation of released chromium for typical ferrochromium alloys available on the global market and compared with the behavior of particles of stainless steel, pure Fe, and pure Cr.

Generated data is essential for the assessment of potential adverse health risks associated with human exposure of ferrochromium particles, performed in parallel by the Finnish Institute of Occupational Health, Finland. This publication will be followed by a study focusing on in vitro exposure scenarios of relevance for skin and eye contact and a study covering health aspects of human exposure to ferrochromium alloys.

Based on in vitro bioaccessibility data from an inhalation and possible ingestion perspective, the following main conclusions are drawn.

The bulk composition of ferro-chromium alloys cannot be used to assess the surface oxide composition primarily composed of chromium(III)oxides, iron(II, III)oxides and silica. The relative proportion of elements in surface oxides depends on prevailing environmental conditions and the degree of crushing and milling to finer-sized particles.

Ferro-chromium alloy particles have surface oxides of passive properties which efficiently limit the extent of metal release in all synthetic biological media investigated. Only a very small fraction, less than 0.3%, of the amount of particles loaded was dissolved and released as metals into solution.

The passive properties of the surface oxides on ferro-chromium alloys improved with time as a result of aging increasing the relative chromium content on the surface, a phenomenon in particular pronounced at acidic conditions.

Neither the surface composition (Cr/(Cr+Fe) ratio of 0.80) nor the bulk composition (Cr/(Cr+Fe) ratio of 0.73) can be used to assess the extent of metals (Cr/(Cr+Fe) ratio varying between 0.06 and 0.12 after 168 h) released from ferro-chromium alloys. Iron was preferentially released compared with Cr for all time periods and media of varying pH and composition investigated. Both elements revealed a time-dependence with decreasing release rates with time.

Released amounts of Fe and Cr were slightly enhanced at acidic condition compared with more pH-neutral conditions of the synthetic media investigated, however, these were very low in all cases, with release rates less than 1.4 and 0.11 $\mu g/cm^2$/week for Fe and Cr, respectively after 1 week of in vitro testing.

Minor elements, present in the alloy in quantities exceeding 0.1 wt%, including V, Mn, and Ni, were released to only a very low extent. Concentrations of V were below the limit of detection and the concentration of Mn less than 10 $\mu g/L$ for all media investigated. Similar conclusions were drawn for Ni being released at a rate of less than 0.03 $\mu g/cm^2$ $week^{-1}$ after 1 week of exposure in gastric fluid (pH 1.5) and even lower in artificial lysosomal fluid (pH 4.5) of relevance for an inhalation scenario.

The release of Fe from the ferro-chromium alloys investigated was significantly lower (approximately 25 times) compared with the release from particles of pure Fe exposed at identical conditions. Compared with AISI 316L stainless steel, the release of Fe was 10 times higher due to surface oxides of less protective properties. Small and comparable amounts of Cr were released from particles of the ferro-chromium alloys, stainless steel AISI 316L, and pure Cr.

The extent of metals released from fine-sized (milled) particles compared with larger-sized particles of the ferro-chromium alloys, pure Fe, and pure Cr was generally slightly higher from fine-sized particles after 1 week of exposure to artificial lysosomal fluid. Independent of size fraction, the extent of particle dissolution was still very low, less than 1.2%.

Chemical speciation measurements clearly revealed Cr to be released as chromium(III) species and not as active (toxic) chromium(VI) species from either the ferro-chromium alloys, the stainless steel AISI 316L, or pure Cr particles for all synthetic media investigated. Except for artificial lysosomal fluid, ALF, containing significant amounts of organic chemicals, all synthetic biological media showed a low capacity to form complexes with released Cr. In ALF, close to 100% of Cr released at these low Cr concentrations was complexed to organic ligands, and not in a potentially bioavailable chemical form.

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REFERENCES

[ASTM] American Society for Testing and Materials. 2003. Standard test method for determining extractability of metals from art materials. In: ASTM. Annual book of ASTM standards, Volume 06. 02. Philadelphia (PA): ASTM, D5517-03.

Balásházy I, Németh I, Alföldy B, Szabó PP, Hegedus CS, Hofmann W, Pálfalvi J, Fehér I, Török S. 2000. Aerosol deposition modeling in human airways and alveoli. *J Aerosol Sci* 31: 482.

[CEN] European Committee for Standardization. 1998. Reference test method for release of nickel from products intended to come into direct and prolonged contact with the skin. Brussels, Belgium: EC Directive 94/27/EC, EN 1811.

Chandler KA, Hudson JC. 1994. Iron and steel. In: Shreir LL, Jarman RA, Burstein GT, editors. Corrosion 3rd ed. Oxford, UK: Butterworth Heinemann, ch 3.1.

Chastain J, King RC. 1995. Handbook of X-ray photoelectron spectroscopy. A reference book of standard spectra for identification and interpretation of XPS data. Eden Prairie (MN): Perkin-Elmer, Physical Electronics Division. 261 p.

Chiba A, Sakakura S, Kobayashi K, Kusayanagi K. 1997. Dissolution amounts of nickel, chromium and iron from SUS 304, 316 and 444 stainless steels in sodium chloride solutions. *J Mater Sci* 32: 1995.

[ChemSec] The International Chemical Secretariat. 2009. The SIN (Substitute It Now!) list 1.0. [cited 2009 September 18]. Available from: http://www.chemsec.org/list

[EC] European Council. 2006. Dangerous substances directive 67/548/EEC Annex I. [cited 2009 September 18]. Available from: http://edexim.jrc.ec.europa.eu/.

[EC] European Council. 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). *Official Journal of the European Union* L396: L136/133–L136/280.

Flint GN, Carter SV, Fairman B. 1998. Skin allergy from exposure to alloys of chromium. *Contact Dermatitis* 39: 315–316.

Hamel SC, Buckley B, Lioy PJ. 1998. Bioaccessibility of metals in soils for different liquid to solid ratios in synthetic gastric fluid. *Environ Sci Technol* 32: 358.

Hanawa T. 2004. Metal ion release from metal implants. *Mater Sci Eng C* 24: 745.

Herting G, Odnevall Wallinder I, Leygraf C. 2005. A comparison of release rates of Cr, Ni, and Fe from stainless steel alloys and the pure metals exposed to simulated rain events. *J Electrochem Soc* 152: B23.

Herting G, Odnevall Wallinder I, Leygraf C. 2006. Factors that influence the release of metals from stainless steels exposed to physiological media. *Corr Sci* 48: 2120.

Herting G, Odnevall Wallinder I, Leygraf C. 2007. Metal release from various grades of stainless steel exposed to synthetic body fluids. *Corr Sci* 49: 103.

Herting G, Odnevall Wallinder I, Leygraf C. 2008a. Corrosion-induced release of chromium and iron from ferritic stainless steel grade AISI 430 in simulated food contact. *J Food Eng* 87: 291.

Herting G, Odnevall Wallinder I, Leygraf C. 2008b. Corrosion-induced release of the main alloying constituents of manganese-chromium stainless steels in different media. *J Environ Monit* 10: 1084–1091.

Herting G, Odnevall Wallinder I, Leygraf C. 2008c. Metal release rate from AISI 316L stainless steel and pure Fe, Cr and Ni into a synthetic biological medium - a comparison. *J Environ Monit* 10: 1092–1098.

Herting G. 2008. Bioaccessibility of stainless steels: Importance of bulk and surface features [Doctoral Thesis]. Stockholm, Sweden: Royal Institute of Technology, KTH.

Herting G, Lindström D, Odnevall Wallinder I, Leygraf C. 2009. Multi-analytical investigation of stainless steel grade AISI 420 in simulated food contact. *J Food Eng* 93: 23.

Hultquist G, Leygraf C, Brune D. 1984. Quantitative measurements of passive dissolution of chromium, iron, and molybdenum from a stainless steel. *J Electrochem Soc* 131: 1773.

[ISO] International Organisation for Standardization. 1995. Air quality - Particle size fraction definitions for health-related sampling. Geneva, Switzerland. ISO 7708.

Keddam M. 1995. Anodic dissolution. In: Marcus P, editor. Corrosion mechanisms in theory and practice 2nd ed. Paris, France: Marcel Dekker Inc., p 97–170.

Kuhn AT, Rae T. 1988. Synthetic environments for the testing of metallic biomaterials. In: Francis PE, Lee TS, editors. The use of synthetic environments for corrosion testing, ASTM STP 970. Philadelphia (PA): American Society for Testing and Materials, p 79–97.

Lesko A, Navara E. 1996. Microstructural characterization of high-carbon ferrochromium. *Mater Charact* 36: 349–356.

Leygraf C, Hultquist G, Olefjord I, Elfström BO, Knyazheva VM, Plaskeyev AV, Kolotyrkin YM. 1979. Selective dissolution and surface enrichment of alloy components of passivated Fe18Cr and Fe18Cr3Mo single crystals. *Corr Sci* 19: 343.

Lide DR. 1999. Handbook of chemistry and physics. 80th ed.ISBN 0-8493-0480 -6. Boca Raton (FL): CRC Press.

Martonen TB, Zhang Z, Yang Y. 1992a. Extrapolation modelling of aerosol deposition in human and laboratory rat lungs. *Inhalation Toxicol* 4: 303–324.

Martonen TB, Zhang Z, Yang Y. 1992b. Interspecies modeling of inhaled particle deposition patterns. *J Aerosol Sci* 23: 389.

Metrohm. 2009. Voltammetric determination of chromium in small quantities. Application bulletin 116. [cited 2009 September 18]. Available from: http://products.metrohm.com/Applications/ab-application-bulletins/ab_101-200/prod-AB-116.aspx

Midander K, Pan J, Leygraf C. 2006. Elaboration of a test method for the study of metal release from stainless steel particles in artificial biological media. *Corr Sci* 48: 2855.

Midander K, Odnevall Wallinder I, Leygraf C. 2007. Metal release from stainless steel particles in vitro — influence of particle size. *J Environ Monit* 9: 74–81.

Miki M, Isawa T, Teshima T, Anazawa Y, Motomiya M. 1992. Difference in inhaled aerosol deposition patterns in the lungs due to three different sized aerosols. *Nucl Med Commun* 13: 553–562.

Moss OR. 1979. Stimulants of lung interstitial fluid. *J Health Phys Soc* 36: 447.

Norlin A, Pan J, Leygraf C. 2002. Investigation of interfacial capacitance of Pt, Ti and TiN coated electrodes by electrochemical impedance spectroscopy. *Biomol Eng* 19: 67.

Odnevall Wallinder I, Bertling S, Berggren Kleja D, Leygraf C. 2006. Corrosion-induced release and environmental interaction of chromium, nickel and iron from stainless steel. *Water, Air, Soil Pollut* 170: 17–35.

Okazaki Y, Gotoh E. 2005. Comparison of metal release from various metallic biomaterials in vitro. *Biomaterials* 26: 11.

Skeaff JMH, James M, Hardy DJ, King P. 2008. A new approach to the hazard classification of alloys based on transformation/dissolution. *Integr Environ Assess Manag* 4: 75–93.

Stockmann-Juvala H, Zitting A, Darrie G, Odnevall WI, Santonen T. 2009. Read-across approach in the risk assessment of ferrochromium. Case: Repeated dose toxicity. *Toxicol Lett* 189: S245.

Stopford W, Turner J, Cappellini D, Brock T. 2003. Bioaccessibility testing of cobalt compounds. *J Environ Monit* 5: 675–680.

Summer B, Fink U, Zeller R, Rueff F, Maier S, Roider G, Thomas P. 2007. Patch test reactivity to a cobalt-chromium-molybdenum alloy and stainless steel in metal-allergic patients in correlation to the metal ion release. *Contact Dermatitis* 57: 35–39.

Sundberg R, Jerlerud-Perez R. 2007. Dissolution/transformation tests of some epoxy mounted copper alloy samples–metallographic study after 7 days exposure. Västerås, Sweden: Scandinavian Copper Development Association. Internal report.

Teixeira F, Berjoan R, Peraudeau G, Perarnau D. 2005. Solar preparation of SiOx ($x \approx 1$) nanopowders from silicon vaporisation on a ZrO2 pellet. XPS and photoluminescence characterisation. *Sol Energy* 78: 763.

Ullmann Y. 2009. Fe- and Cr-based metal particles. Bioaccessibility studies from a human health and environmental perspective [M.S. Thesis]. Stockholm, Sweden: Royal Institute of Technology, KTH.

[UN] United Nations. 2003. The globally harmonized system of classification and labelling of chemicals. ST/SG/AC.10/30/Rev. 1. [cited 2009 September 18]. Available from: http://www.unece.org/trans/danger/publi/ghs/ghs_rev01/English/00e_intro.pdf

[UN] United Nations. 2005. The globally harmonized system of classification and labelling of chemicals, ST/SG/AC, 10/30/Rev. 1. [cited 2009 October 28]. Available from: www.unece.org/trans/danger/publi/ghs/ghs_rev01/01files_e.html