



Testing in artificial sweat – Is less more? Comparison of metal release in two different artificial sweat solutions



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ABSTRACT

Metal release from materials immersed in artificial sweat can function as a measure of potential skin exposure. Several artificial sweat models exist that, to various degree, mimic realistic conditions. Study objective was to evaluate metal release from previously examined and well characterized materials in two different artificial sweat solutions; a comprehensive sweat model intended for use within research, based on the composition of human sweat; and the artificial sweat, EN1811, intended for testing compliance with the nickel restriction in REACH. The aim was to better understand whether there are advantages using either of the sweat solutions in bio-elution testing of materials. Metal release in two different artificial sweat solutions was compared for discs of a white gold alloy and two hard metals, and a rock drilling insert of tungsten carbide at 1 h, 24 h, 1 week and 1 month. The released amount of metal was analysed by means of inductively coupled plasma mass spectrometry. Similar levels of released metals were measured from test materials in the two different artificial sweat solutions. For purposes in relation to legislations, it was concluded that a metal release test using a simple artificial sweat composition may provide results that sufficiently indicate the degree of metal release at skin contact.

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1. Introduction

Daily use of objects and products made from pure metals, alloys and other metal-containing materials often involves skin contact that may result in deposition of metals on the skin (Erfani et al., 2015; Julander et al., 2009, 2013; Midander et al., 2014). Skin exposure to metals can cause local effects, in particular contact allergy and allergic contact dermatitis to the metals nickel, cobalt or chromium. Skin may also function as a transport route for further exposure to metals systemically (Franken et al., 2015).

When a metal-containing material is in contact with the skin, the material surface is affected by sweat present on the skin surface resulting in release of metal from the material due to dissolution, corrosion and/or wear-processes (Erfani et al., 2015; Julander et al., 2013). The extent of metal release depends on specific conditions at contact, related to skin, material and environment. Such conditions include duration and frequency of contact, temperature, pH, presence of sweat, sebum and other skin components, as well as

material properties such as type of material (pure metal, alloy, other materials with metal-containing surface coating or metal incorporated in the matrix) and condition of the surface (Girod et al., 2012; Taylor and Machado-Moreira, 2013).

With regards to nickel allergy, the release of nickel from materials immersed in artificial sweat can function as a measure for assessment of potentially harmful skin exposure (Thyssen et al., 2011). This idea illustrates the concept of bio-elution testing by *in vitro* methods measuring release of a substance or metal into artificial biological fluids that, to some degree, simulate a realistic exposure scenario (Midander, 2009). The result of a release test in artificial sweat at defined conditions mimicking skin contact, may be considered a “worst case scenario” under the assumption that *all* the nickel released from a material surface in contact with skin (sometimes referred to as the *bioaccessible* fraction of the metal release) will deposit *onto* the skin. Data on bioaccessible metal that is potentially available for skin absorption and following systemic or local effects, can be used for risk assessment and protection against potential harmful skin contact. However, the experimental limitations, such as various complexity of the artificial sweat and analytical compromises, need to be taken into account. This is the case in the restriction of nickel release from items that are intended for prolonged contact with the skin in REACH (The Commission of

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the European Communities, 2009). It is however important to note that the bioaccessible metal fraction, an *in vitro* measure, is not necessarily equal to either the amount of metal that deposits on skin at contact (*the skin dose*) or the *bioavailable* fraction that is absorbed into the skin, which are results of exposure *in vivo*.

The most widely used formulation of artificial sweat was described by Pedersen et al. (1974) as a test solution with a pH buffered to 6.5, mimicking the salt content or ionic strength of human sweat. This artificial sweat composition was adopted, and the test conditions were specified, in the European reference method EN1811, approved in 1998 and later slightly revised (European Committee for Standardization (CEN), 2011), for test of compliance with the nickel restriction in REACH. Ever since, this artificial sweat solution has been used to assess release of nickel and other metals from various materials and items with an intended use resulting in skin contact (Hamann et al., 2015; Hedberg et al., 2010; Kettelarjij et al., 2014; Kulthong et al., 2010; Lidén et al., 1998; Rezić et al., 2009; Thyssen et al., 2010b). In the test protocol of EN1811, a white gold alloy is defined as a quality control material of the method, based on results from clinical and laboratory testing by CEN/TC283/WG4 in the development of EN1811 (Lidén et al., 1996). The main feature of this reference material is that it releases nickel at the level of the limit value in the restriction; 0.5 $\mu\text{g}/\text{cm}^2/\text{week}$, upon immersion under the conditions of EN1811.

Other artificial sweat formulations exist, such as the ISO artificial sweat for test of “color fastness” to perspiration (ISO Textiles, 1994) at pH 5.5 or 8, and the sweat in the American Association of Textile Chemists and Colorists test method (aimed for similar purposes) at pH 4.3 (AATCC Test method 15–2002). These sweat formulations have for example been used for assessment of silver nanoparticle release from antibacterial fabrics (Kulthong et al., 2010; von Goetz et al., 2013).

There are also sweat models that are not aimed for compliance testing of regulatory issues. These sweat models are usually more complex and based on the constituents of human sweat, hence realistic test conditions can be further mimicked. Callewaert et al. recently assembled an artificial sweat composition including human fatty acids (pH 6.5) that was primarily designed to mimic human axillary sweat for studies of cosmetics and malodour (Callewaert et al., 2014). A comprehensive artificial sweat model based on the composition of human sweat has also been developed, tested and used in several studies (Harvey et al., 2010; Stefaniak et al., 2014a; Stefaniak and Harvey, 2006).

The influence of different sweat components (organic- or inorganic substances such as salts, amino acids, fatty acids, vitamins etc.) on metal release from materials has been investigated partly. For example, sebum lipids are considered having a limited effect on metal release while the addition of amino acids may promote release due to their ability to complex metal ions as in the case of cysteine and gold (Stefaniak and Harvey, 2006). A structured study on the influence of artificial sweat components (EN1811) on metal release from a copper-nickel alloy provides more information on how for example pH and constituents of the sweat solution affect corrosion and metal release (Brugnoni et al., 2005). Comparison of release in different sweat compositions have been performed in some studies of silver release from antibacterial fabrics and for hard metal powders (Kulthong et al., 2010; Stefaniak et al., 2010b; von Goetz et al., 2013).

In this study we compare metal release from well-characterized test materials in two different artificial sweat solutions. A comprehensive sweat model intended for use within research and based on the composition of human sweat, and the artificial sweat, EN1811, intended for testing compliance with the nickel restriction in REACH were used. The aim of this paper is to better understand whether there are advantages using either of the sweat solutions in

bio-elution testing.

2. Materials and methods

White gold discs 18 K (Au 75.89%, Cu 16.01%, Ni 6.00%, Zn 2.10%; surface area 2.4 cm^2/disc), quality control material in EN 1811:2011, were purchased from Allgemeine Gold und Silberscheideanstalt AG, Pforzheim, Germany. One day prior to starting immersion experiments the discs were abraded with wet emery paper (SiC; 600 grit followed by 1200 grit), rinsed with deionized water ($16.7 \text{ M}\Omega\text{cm}^{-1}$), degreased in 0.5% sodium dodecylbenzene sulfate solution (2 min), rinsed again until there was no foaming, gently dried with paper tissue, and placed on paper tissue to dry overnight.

Hard metal (cemented tungsten carbide) discs of two different compositions were used; 6% Co, and 15% Co and 0.6% Cr, respectively. The discs were manufactured and provided by Sandvik AB (Sandviken, Sweden). Discs of the same materials and batches have previously been studied (Julander et al., 2009; Midander et al., 2014; Thyssen et al., 2010a). The diameter of each of the discs was ~11 mm, and the total surface area was calculated to be 2.7 cm^2 each (Julander et al., 2009). The surfaces of the hard metal discs were used in as-received condition and were rinsed and degreased prior to experiments, according to the procedure described above.

Hard metal rock drilling inserts (buttons) were provided by Sandvik Tooling Sverige AB (Västberga, Sweden). The inserts contained 6% Co with trace amounts of nickel and chromium. The surface area was calculated to 3.3 $\text{cm}^2/\text{insert}$. The surface was non-coated and the inserts were used in as-received condition, rinsed and degreased prior to experiments.

All glass and plastic utensils were acid cleaned in 10% HNO_3 prior to use (soaked in 10% HNO_3 for 24 h, then rinsed 4 times in deionized water and dried in ambient air in the laboratory). Nitrile or vinyl gloves were used throughout all the preparations of samples and solutions and during the manual procedures of the immersion experiments. Spatulas, spoons and weighing funnels were cleaned with deionized water and ethanol (95%) and dried with laboratory paper tissues in between weighing of solid chemicals.

2.1. Artificial sweat solutions

Artificial sweat was prepared according to the reference test method EN 1811:2011 on the same day as the immersion experiments started by mixing urea (0.1 wt%), NaCl (0.5 wt%) and lactic acid (0.1 wt%) in deionized water. A Sartorius balance (Sartorius MPact AX423 with internal calibration, Göttingen, Germany) was used for weighing of the chemicals. The pH of the solution was adjusted with 1 M NaOH, 0.1 M NaOH and 0.1 M HCl to a final pH of 6.5 ± 0.05 (Mettler Toledo Seven Easy Inlab[®] Expert Pro pH, Schwerzenbach, Switzerland).

Preparation for making the comprehensive artificial sweat, using a low vitamin formulation (advice by personal communication, Aleksandr Stefaniak, CDC/NIOSH USA, November 2011), took place during several days.

The amounts of some chemical constituents were too small to be accurately weighted, hence stock solutions, denoted A-U, were prepared according to description in Table 1. The chemicals for stocks were weighted using a Sartorius AX423 balance.

The solid chemicals (Table 1) for the comprehensive sweat preparation were weighted directly in 1.5 mL Eppendorf tubes (Sarstedt, Nümbrecht, Germany) using a stainless steel spatula, on a balance using the four decimal mode (Sartorius Research R160P with internal calibration, Sartorius, Göttingen, Germany).

Deionized water for the comprehensive artificial sweat was preheated to $37 \pm 2 \text{ }^\circ\text{C}$ in a heating cabinet (Memmert, Schwabach, Germany) overnight before mixing the comprehensive artificial

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