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Genotoxicological assessment of two reactive dyes extracted from cotton fibres using artificial sweat

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ABSTRACT

Human eyes have a remarkable ability to recognize hundreds of colour shades, which has stimulated the use of colorants, especially for clothing, but toxicological studies have shown that some textile dyes can be hazardous to human health. Under conditions of intense perspiration, dyes can migrate from coloured clothes and penetrate into human skin. Garments made from cotton fabrics are the most common clothing in tropical countries, due to their high temperatures. Aiming to identify safe textile dyes for dyeing cotton fabrics, the genotoxicity [*in vitro* Comet assay with normal human dermal fibroblasts (NHDF), Tail Intensity] and mutagenicity [*Salmonella*/microsome preincubation assay (30 min), tester strains TA98, TA100, YG1041 and YG1042] of Reactive Blue 2 (RB2, CAS No. 12236-82-7, C.I. 61211) and Reactive Green 19 (RG19, CAS No. 61931-49-5, C.I. 205075) were evaluated both in the formulated form and as extracted from cotton fibres using different artificial sweats. Both the dyes could migrate from cotton fibres to sweat solutions, the sweat composition and pH being important factors during this extraction. However, the dye sweat solutions showed no genotoxic/mutagenic effects, whereas a weak mutagenic potential was detected by the Ames test for both dyes in their formulated form. These findings emphasize the relevance of textile dyes assessment under conditions that more closely resemble human exposure, in order to recognize any hazard.

1. Introduction

Synthetic dyes are extensively used in many fields of up-to-date technology, such as the textile, food and cosmetic industries (Forgacs et al., 2004; Ferraz et al., 2011). With respect to textile dyes, there is insufficient information about their potential health risks for consumers (Brüschweiler et al., 2009). The toxicological data available for these chemicals have shown effects that range from contact allergies to a variety of genetic damage (Jäger et al., 2004; Schneider et al., 2004; Moreau and Goossens, 2005; Malachová et al., 2006; Novotný et al., 2006; Klemola et al., 2007; Tsuboy et al., 2007; Brookstein, 2009; Lademann et al., 2009; Meinke et al., 2009; Ryberg et al., 2009; Chequer et al., 2009, 2011; Ferraz et al., 2010, 2011; Oliveira et al., 2010).

The use of clothing promotes direct contact of the textile dyes with the human skin (Brookstein, 2009). Despite the skin has the function to protect the body against toxic chemicals (*stratum corneum* barrier) (Katritzky et al., 2006), studies have shown that some textile dyes can migrate from fabrics and penetrate into the human skin in cases of perspiration (Brookstein, 2009; Lademann et al., 2009; Meinke et al., 2009). Heretofore, the concern about the fact that textile dyes could penetrate through the skin was focused basically on cases of contact allergies and dermatitis (Moreau and Goossens, 2005; Brookstein, 2009; Lademann et al., 2009; Meinke et al., 2009; Ryberg et al., 2009). However, once a toxic dye has entered the human body via the skin, it can also induce other kinds of harmful effects to the exposed cells and possible deleterious effects include DNA damage, which is related to several diseases, including cancers (Speit, 2009; Lord and Ashworth, 2012).

Tropical countries require garments made from soft fabrics such as cotton, due to the high temperatures that occur throughout almost the whole year. Cotton accounts for about 40% of the world fibre production (Klemola et al., 2007) and can be coloured using reactive dyes (Fang et al., 2013). The reactive dyes, which are so called due to the method of application, differ from the other classes of dyes since they are covalently bound to the textile fibres (Akar et al., 2009). In addition, the popularity of reactive dyes is

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related to several advantages, such as bright colours, excellent colour fastness, water-fastness and simple application techniques (Akkaya et al., 2007). The reactive dyeing method uses large amounts of electrolytes (e.g. sodium chloride), which are necessary for absorption of the dyestuff by the fibre material, and of alkali metal hydroxides (e.g. sodium hydroxide), that act as fixing alkalis (Periyasamy et al., 2011). In some cases an additional rinsing treatment can also be employed, aiming to increase the fastness of the coloured fabrics (Âlcantara and Daltin, 1996).

Within this context, the identification of safe textile dyes for colouring cotton textiles is relevant to avoid health problems to humans exposed to colourful garments. Thus, the present work assessed the migration rate of Reactive Blue 2 (RB2; anthraquinone dye; CAS No. 12236-82-7; C.I. 61211; 1-Amino-4-[[4-[[4-chloro-6-[[3 (or 4)-sulfophenyl]amino]-1,3,5-triazin-2-yl]amino]-3-sulfophenyl]amino]-9,10-dihydro-9,10-dioxo-2-anthracenesulfonic acid) and Reactive Green 19 (RG19; azo dye; CAS No. 61931-49-5; C.I. 205075; 2,7-Naphthalenedisulfonicacid,4-amino-3,6-bis[2-[4-[[4-chloro-6-[(3-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-2-sulfophenyl]diazenyl]-5-hydroxy-,hexasodium salt), extracted using different artificial sweats from cotton fabrics which were coloured by two dyeing processes – with and without a rinsing treatment. The dye sweat solutions that showed the highest extracted concentration were toxicologically evaluated regarding their potential to induce DNA damage. The Alkaline Comet assay with *in vitro* human dermal fibroblasts and the *Salmonella* mutagenicity assay were the genetic toxicological tests chosen for this investigation. Using these tests the authors were able to evaluate the genotoxic [DNA lesions, such as DNA single-strand breaks (SSB), alkali-labile sites, DNA–DNA/DNA-protein cross-linking, and SSB associated with incomplete excision repair sites] (Tice et al., 2000) and mutagenic (gene mutations, such as base-pair substitution and frameshifts) (Mortelmans and Zeiger, 2000) activities of these dyes.

2. Material and methods

2.1. Dyes and textile

The textile dyes, RB2 (CAS No. 12236-82-7, dye content 60%) and RG19 (CAS No. 61931-49-5, dye content 65%), all from Sigma-Aldrich, were analyzed regarding their hazard to human health (Fig. 1). Phosphate buffered saline (PBS) was used as the vehicle to prepare the formulated dye solutions. 100% cotton fabric was used for textile dyeing (Fiação e Tecelagem São Geraldo, Contagem-MG, Brazil).

2.2. Textile dyeing

The cotton fabrics were dyed according to Neves and Crespim (2000), with slight modifications. Prior to the textile dyeing, the cotton fabric was washed in water, aiming to eliminate possible finishes that could be present in the cotton fabric used. Briefly, small pieces of the textile (3×6 cm^2) were prepared, weighed and placed into dye baths with a liquid ratio of 1:30 (1 kg textile/30 L water) at 50 °C. The dyes RB2 and RG19 were then added to their respective dye baths in amounts based on the textile weight (2% of fabric weight). An electrolyte, used for dye absorption, was added to the dye bath during the first 20 min in the proportion of 70 g sodium chloride/L water. 40 min after the start, an alkali (15 g sodium carbonate/L water) was also added to the dye bath to promote dye fixation. The pH was then adjusted to $\geqslant 11$ with sodium hydroxide and the textiles incubated for a further 1 h in the dye liquor. A cold water wash was finally used to remove unfixed dyes from the dyed fabrics. Half of the dyed fabrics were air dried and stored at room temperature until the sweat extraction. The remaining part was submitted to an additional washing step as follows: 5 min in water at 60 °C; 15 min in a bath with a colloid dispersant (0.6 g of Resiwash AC/L water – Resinac, Jandira-SP/Brazil) at boiling temperature; 3 min in water at 60 °C and then finally 3 min of cold water washing.

2.3. Artificial sweat and dye extraction

Three different solutions of artificial sweat were prepared according to the International Standard Organization (ISO 105-E04-2008E) and the British Standard (BS EN1811-1999) (as cited in Kulthong et al., 2010) and used as extracting agents (Table 1).

The dye extractions were carried out according to Meinke et al. (2009) as follows: pieces of the coloured cotton fabrics were weighed and extracted with a liquid ratio of 1:50 (20 mg textile/mL extracting agent) at 37 °C and 42 °C. Sweat samples were collected for each sweat pH value (5.5, 6.5 and 8.0) at two different time points: 2 and 8 h.

2.4. Chromatographic analysis to determine the dye concentration in the artificial sweat samples

Prior to chemical analysis, the sweat samples underwent solid phase extraction using a Strata™-X 33 µm cartridge (Polymeric Reversed Phase, 200 mg/3 mL, Phenomenex) and were eluted using aqueous acetonitrile (ACN/H_2O, 50:50 – v/v). The extracts obtained

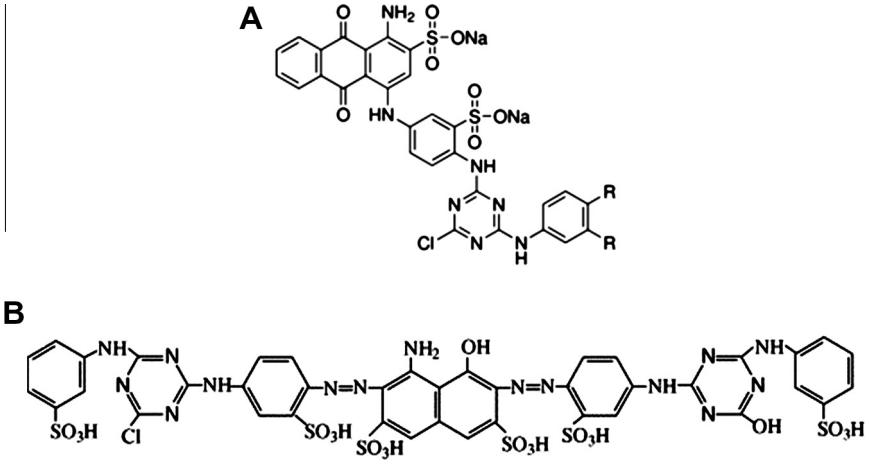

Fig. 1. The chemical structures of the textile dyes studied. (A) Reactive Blue 2 (anthraquinone dye) and (B) Reactive Green 19 (azo dye).

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