Regulatory Toxicology and Pharmacology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Identification of non-regulated aromatic amines of toxicological concern which can be cleaved from azo dyes used in clothing textiles

Beat J. Brüschweiler [a,*], Simon Küng [b], Daniel Bürgi [b], Lorenz Muralt [c], Erich Nyfeler [c]

[a] Federal Food Safety and Veterinary Office, Schwarzenburgstrasse 155, CH-3003 Bern, Switzerland
[b] FRIEDLIPARTNER AG, Nansenstrasse 5, CH-8050 Zürich, Switzerland
[c] Official Food Control Authority of the Canton Bern, Muesmattstrasse 19, CH-3012 Bern, Switzerland

ARTICLE INFO

Article history:
Received 23 February 2014
Available online xxxx

Keywords:
Textile dyes
Carcinogenicity
Genotoxicity
Skin sensitization

ABSTRACT

Azo dyes in textiles may release aromatic amines after enzymatic cleavage by skin bacteria or after dermal absorption and metabolism in the human body. From the 896 azo dyes with known chemical structure in the available textile dyes database, 426 azo dyes (48%) can generate one or more of the 22 regulated aromatic amines in the European Union in Annex XVII of REACH. Another 470 azo dyes (52%) can be cleaved into exclusively non-regulated aromatic amines. In this study, a search for publicly available toxicity data on non-regulated aromatic amines was performed. For a considerable percentage of non-regulated aromatic amines, the toxicity database was found to be insufficient or non-existent. 62 non-regulated aromatic amines with available toxicity data were prioritized by expert judgment with objective criteria according to their potential for carcinogenicity, genotoxicity, and/or skin sensitization. To investigate the occurrence of azo dye cleavage products, 153 random samples of clothing textiles were taken from Swiss retail outlets and analyzed for 22 high priority non-regulated aromatic amines of toxicological concern. Eight of these 22 non-regulated aromatic amines of concern could be detected in 17% of the textile samples. In 9% of the samples, one or more of the aromatic amines of concern could be detected in concentrations >30 mg/kg, in 8% of the samples between 5 and 30 mg/kg. The highest measured concentration was 622 mg/kg textile. There is an obvious need to assess consumer health risks for these non-regulated aromatic amines and to fill this gap in the regulation of clothing textiles.

1. Introduction

Azo dyes are by far the most widely used synthetic organic dyes in textiles (FRIEDLIPARTNER AG, 2009a,b). They can be cleaved by skin bacteria, by dermal or systemic metabolism into aromatic amines which might have undesired toxicological properties, particularly carcinogenic and allergenic potential (Platzek, 2010). According to the European Commission Regulation 552/2009/EC, all azo dyes based on carcinogenic aromatic amines are banned for use in textile and leather articles (EC, 2009a,b). This was implemented in the REACH regulation Annex XVII. Azo dyes which, by reductive cleavage of one or more azo groups, may release one or more of 22 specified aromatic amines in detectable concentrations, i.e. above 30 mg/kg (0.003% by weight) in the articles or in the dyed parts thereof shall not be used in textile articles which may come into direct and prolonged contact with the human skin as clothing, footwear, gloves, hats, etc. In Switzerland this has been regulated in harmonization with the EU in the Ordinance on Materials and Articles with Human Contact (SR 817.023.41) (FDHA, 2005a). The regulation of the 22 aromatic amines is based on the classification of 14 aromatic amines as EU carcinogenic category I or II, and of 8 additional aromatic amines as the previous carcinogenic class A1 or A2 (compounds which cause concern because of demonstrated or potential carcinogenic effects but which cannot be definitely evaluated because of insufficient data) by the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK) (LGC, 1998; CSTEE, 1999). Its origin lies in the second amendment of the German Consumer Goods Ordinance in 1994 that banned the use of certain azo dyes in consumer goods that can potentially come into direct, long-term contact with human skin (BGA, 1994). Since 1999, the European Union had explored the restriction of azo dye usage, and in 2002, it published the 19th amendment to the Council Directive 76/69/EEC (EC, 2002). This EU Directive banned 20 aromatic amines listed in the German Ordinance. Two additional aromatic amines, namely o-anisidine and 4-aminoazobenzene, were also banned at the same

* Corresponding author. Address: Federal Food Safety and Veterinary Office, Risk Assessment Division, Schwarzenburgstrasse 155, CH-3003 Bern, Switzerland.
E-mail address: beat.brueschweiler@blv.admin.ch (B.J. Brüschweiler).

http://dx.doi.org/10.1016/j.yrtph.2014.04.011
0273-2300/

time leading to the regulation of those 22 aromatic amines with a limit value of 30 mg/kg textile.

The motivation behind this study was the fact that a systematic and updated overview on the toxicity of the other non-regulated aromatic amines is missing. For instance, the non-regulated 2,4-xylidine (Chemical Abstracts Service Registry Number (CASRN) 95-68-1), classified by MAK as Carc. Cat. 2, can be cleaved from C.I. Acid Red 26. A comprehensive inventory of non-regulated aromatic amines as cleavage products of known textile azo dyes was compiled. Literature and databases searches were performed for available toxicity data. Based on these toxicity data, priority lists of substances were compiled. 153 samples of clothing textiles were taken from several clothing retail outlets in the Canton of Bern in Switzerland and analyzed for 22 aromatic amines of high priority.

2. Methods

2.1. Data source for textile azo dye inventory

Textile dyes had previously been inventorized in a database using available data sources from dye producers, industrial associations, textile labels, seals of quality, official authorities and academia (FRIEDLIPARTNER AG, 2009a,b). The main criterion for inclusion in the inventory was the availability of a CASRN and/or a Color Index (C.I.) name. Positive and negative lists were collated: the positive list contains dyes which are adequately documented by producers or listed in scientific publications, while the substances in the negative list are banned by existing regulations, or use restricted by product labeling or seals of quality (Supplement Table 1). A comprehensive list of 1980 dye substances could be compiled. The CASRNs could be identified for 1847 (93%) and the chemical structures for 1584 (80%) of the substances.

2.2. Literature search for CASRN and toxicity data

Based on the chemical structures of the azo dyes in the textile dyes database (FRIEDLIPARTNER AG, 2009b) all possible cleavage products were generated using the structure drawing software ChemDraw (CambridgeSoft Version 12.0, 2009) by cleaving all azo bonds into amines. In a next step, we tried to identify the CASRN of the aromatic amines by using CWM Global Search (Akos GmbH, D) and STN (FIZ Karlsruhe, D). The regulated aromatic amines were identified by CASRN and their parent azo dyes sorted out. For the non-regulated aromatic amines, a toxicity data search was performed for substances with identified CASRN in Toxnet (Toxicology Data Network by the US National Library of Medicine), ChemIDplus Lite (US National Library of Medicine) and GESTIS (Information system on hazardous substances of the German Social Accident Insurance). For a selected number of aromatic amines, a search was also performed in RTECS (Registry of Toxic Effects of Chemical Substances). Relevant datasets were also sought in CAMEO Chemicals (US National Oceanographic and Atmospheric Administration), CCRIS (Chemical Carcinogenesis Research Information System in Toxnet), GENETOX (Genetic Toxicology Data Bank in Toxnet), HSDB (Hazardous Substances Database in Toxnet), IUCLID (International Uniform Chemical Information Database) and NTP (National Toxicology Program of the National Institute of Environmental Health Sciences, NIEHS). GESTIS contained useful information on EU classification and labeling. The data search included toxicological endpoints with possible relevance when wearing clothes and having skin contact: carcinogenicity, genotoxicity, skin sensitization, irritation, reproductive and developmental toxicity. In addition, all relevant EU risk phrases from the GESTIS database and the toxicity data from RTECS were collected.

2.3. Toxicity scoring system and priority substance lists

For the prioritization of the azo dyes, an own new toxicity scoring system was established (Supplement Table 2). As toxicological endpoints, acute toxicity, organ toxicity, subacute/subchronic/chronic toxicity, skin corrosion, irritation, sensitization/allergenicity, genotoxicity, carcinogenicity, and reproductive/developmental toxicity were considered. For each toxicological endpoint, criteria were evaluated to allocate a value of 3 (high toxicity), 2 (medium toxicity), 1 (low toxicity) or "?" (no data available). As criteria, EU classification, IARC classification as well as additional information from RTECS and Toxnet were taken into account. Substances with the maximum toxicity score of 3 were regarded as high priority, of 2 as medium priority, and of 1 as low priority. For the substances with a score of 3, a more detailed literature search was performed in monographs, opinions, evaluations, etc. of the International Agency for Research on Cancer (IARC), the National Toxicology Program (NTP), and the US Environmental Protection Agency (US EPA). Two different priority lists were compiled, one for the toxicological endpoints carcinogenicity and/or genotoxicity, and one for sensitization by skin contact.

2.4. Chemical analysis

153 samples of colored or black clothing were bought in major supermarkets and clothes stores in the Canton of Bern. The most important criteria for the selection of the samples were direct contact with skin, possible contact with sweat, and contact with sensitive skin. The selected samples were mainly T-shirts, underwear, sport clothes, scarves and clothes for children.

Clothes with different colors were first analyzed as mixed samples with all colored parts together. From samples with positive results, defined as concentrations of aromatic amines above 5 mg/kg, the colored parts of the clothes were again analyzed separately. In this way, it was possible to assign the aromatic amines detected to particular colors and parts of the clothes.

The aromatic amines were extracted from the samples using a method based on DIN EN 14362-1 with minor modifications (DIN, 2012). A representative part of the sample was cut into pieces of about 25 mm^2 size. 500 mg of this material was transferred to a 50 ml polypropylene centrifuge tube and mixed with 8.5 ml of citrate buffer (pH 6) which was preheated to 70 ± 2 °C. The tube was closed, shaken gently and placed in a water bath at 70 °C for 30 min. 1.5 ml of aqueous sodium dithionite solution was added and the resulting mixture was kept for 30 min at 70 °C. The reaction vessel was rapidly cooled down to room temperature using cold water. 20 ml of methanol were added and the tube was ultrasonicated for 10 min. The tube was filled up with ultrapure water to the mark of 50 ml, then the mixture was centrifuged at 4000 rpm for 5 min. The supernatant was used for the analysis either directly or after filtration using a 0.22 µm membrane filter.

The extracts were analyzed by LC–MS/MS using an Ultimate 3000 HPLC from Dionex and a QTrap 3200 from ABSciex. 5 µl of extract were injected onto a Thermo-Dionex Acclaim Polar Advantage II 3 µm 150 × 2 mm HPLC column using an ammonium formate buffer (5 mmol, pH 4.5) containing 5% acetonitrile at a flow rate of 0.3 ml/min. A 1% solution of formic acid in acetonitrile was added after the chromatography step to obtain a more sensitive ionization process in the mass spectrometer. For the ionization, a turbo spray method was used, either in negative or in positive mode depending on the character of the different aromatic amines. The limits of detection ranged from 0.05 to 0.5 mg/kg textile and the limits of quantification from 0.2 to 2 mg/kg.

Please cite this article in press as: Brüschweiler, B.J., et al. Identification of non-regulated aromatic amines of toxicological concern which can be cleaved from azo dyes used in clothing textiles. Regul. Toxicol. Pharmacol. (2014), http://dx.doi.org/10.1016/j.yrtph.2014.04.011