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Azo dyes in clothing textiles can be cleaved into a series of mutagenic aromatic amines which are not regulated yet

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ABSTRACT

Azo dyes represent the by far most important class of textile dyes. Their biotransformation by various skin bacteria may release aromatic amines (AAs) which might be dermally absorbed to a major extent. Certain AAs are well known to have genotoxic and/or carcinogenic properties. Correspondingly, azo dyes releasing one of the 22 known carcinogenic AAs are banned from clothing textiles in the European Union. In the present study, we investigated the mutagenicity of 397 non-regulated AAs potentially released from the 470 known textile azo dyes. We identified 36 mutagenic AAs via publicly available databases. After predicting their mutagenicity potential using the method by Bentzien, we accordingly allocated them into different priority groups. Ames tests on 18 AAs of high priority showed that 4 substances (22%) (CASRN 84-67-3, 615-47-4, 3282-99-3, 15791-87-4) are mutagenic in the strain TA98 and/or TA100 with and/or without rat S9 mix. Overall, combining the information from the Ames tests and the publicly available data, we identified 40 mutagenic AAs being potential cleavage products of approximately 180 different parent azo dyes comprising 38% of the azo dyes in our database. The outcome of this study indicates that mutagenic AAs in textile azo dyes are of much higher concern than previously expected, which entails implications on the product design and possibly on the regulation of azo dyes in the future.

1. Introduction

Azo dyes represent the most commonly used group of dyes in textile industry (Lacasse and Baumann, 2004; FriedliPartner, 2009a, 2009b; Brüschweiler et al., 2014). They were reported to constitute 60–70% of all dyestuff concerning textile production (Rawat et al., 2016). Dermal, systemic and bacterial biotransformation of azo dyes can release aromatic amines (AAs) (BGFA, 2009; Platzek et al., 1999; Stingley et al., 2010). AAs on the skin might be dermally absorbed to a major extent (Korinth et al., 2013).

AAs are used as intermediates in the synthesis of azo dyes (Weglarz-Tomczak and Gorecki, 2012; Freeman, 2013). As recently reviewed by Platzek (2010), AAs exposures from consumer products bear risks for human health, particularly associated to mutagenic and/or carcinogenic properties of certain AAs. Toxicity of AAs depends on the metabolic activation of the amino group, which can generate the reactive intermediate hydroxylamine known to damage DNA and proteins (Neumann, 2010).

Azo dyes which may release one of the 22 as yet regulated carcinogenic AAs are banned from clothing textiles in the European Union (Annex XVII of the REACH regulation; No, 1907/2006) (EC, 2009) and in national regulations, e.g. in Switzerland in the Ordinance about objects with human contact (SR 817.023.41) (FDHA, 2005). Regulation of these 22 AAs was based on their classification as carcinogens, whereupon 14 were assigned to category I and II according to the previous EU system (today 1A and 1B) and 8 to the previous carcinogenic class A1 and As by the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK) (LGC, 1998; CSTEE, 1999).

From the 896 azo dyes with known chemical structure in our textile dyes database (FriedliPartner, 2009b; Brüschweiler et al., 2014), 426 azo dyes (48%) are potential parent compounds of one or more of the 22 regulated AAs, while the other 470 azo dyes (52%) are exclusively metabolized to non-regulated[1] AAs.

Biotransformation of these 470 azo dyes can release 397

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[1] non-regulated only in the context of clothing textiles and not in the context of other regulations (e.g. CLP).

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different AAs from which Brüschweiler et al. (2014) could compile a priority list with 15 non-regulated AAs suspected mostly to be genotoxic and/or in some cases carcinogenic based on available literature. Following reductive cleavage according to EN 14362-1 (DIN, 2012) performed on 153 samples of clothing textiles that were purchased from clothing retail outlets in Switzerland, four potentially genotoxic and/or carcinogenic substances, 2,2'-dimethylbenzidine, 4-aminophenol, 4-ethoxyaniline and aniline, were detected. Within these analyses amounts up to 588 mg/kg aniline and 134 mg/kg 4-ethoxyaniline were measured in textiles (Brüschweiler et al., 2014; KL BE, 2014).

This study aims at the investigation of the mutagenicity of AAs representing cleavage products of azo dyes used in clothing textiles, since a systematic evaluation is missing so far. In a first step, we applied a modified *in silico* method by Bentzien et al. (2010) to predict Ames activities of primary AAs by calculating the stability of the metabolically intermediate nitrenium ions. Based on the outcome of this analysis and other criteria, we assigned the AAs into different priority groups. We selected 23 AAs and performed experimental Ames tests in the strains TA98 and TA100 with and without metabolic activation to clarify their mutagenicity. Additionally, we queried publicly available relevant databases and literature for relevant experimental Ames test data.

2. Materials and methods

2.1. Dataset

This study refers to an inventory of textile dyes using available data sources from dye producers, industrial associations, textile labels, seals of quality, official authorities and scientific institutions (FriedliPartner, 2009a, 2009b). The database contains 470 azo dyes that can be cleaved into 397 unique, non-regulated[1] AAs (Brüschweiler et al., 2014; this study).

2.2. Sources for mutagenicity data

The available experimental mutagenicity data were taken from the mutagenicity dataset by Kazius et al. (2005), the Chemical Carcinogenesis Research Information System (CCRIS), and the Carcinogenicity Potency Database (CPDB). The CCRIS database contains chemical records with mutagenicity, carcinogenicity, tumor promotion, and tumor inhibition test results. It was developed by the National Cancer Institute (NCI). Data are derived from studies cited in primary journals, current awareness tools, NCI reports, and other sources. Test results have been reviewed by experts in carcinogenesis and mutagenesis. Further important data sources were the informations on chemicals by ECHA and Jung et al. (1992).

2.3. Ames test

The bacterial reverse-mutation screening assay (Ames test) with the *S. typhimurium* strains TA98 and TA100 was designed to be compatible with the procedure indicated in the OECD test guideline No. 471, with and without metabolic activation (S9 mix) (OECD, 1997). The work was conducted by Envigo CRS GmbH (Rossdorf, Germany) following good laboratory practices and adhering to the applicable standard operation procedures. These strains were chosen as they are used in reduced versions of the Ames test because TA98 is capable of detecting frameshift mutations, while TA100 detects base pair substitutions. Harding et al., 2015 could demonstrate the significance of TA98 and TA100 for the detection of AA mutagenicity.

In a first experiment, a plate incorporation assay was performed with all test substances. In the case a borderline result was obtained, a second experiment in form of a pre-incubation assay was performed. The *S. typhimurium* strains TA98 and TA100 were obtained from Trinova Biochem GmbH (Giessen, Germany). All test substances were dissolved in DMSO. As positive control substance without metabolic activation, sodium azide (NaN_3) was used in the strain TA100 and 4-nitro-o-phenylene-diamine (4-NOPD) in the strain TA98. With metabolic activation, 2-aminoanthracene (2-AA) was used a positive control. Concurrent untreated and solvent controls were also performed. For the preparation of the S9-mix, phenobarbital/β-naphthoflavone-induced rat liver S9 was used as the metabolic activation system.

The assay was considered acceptable, if the following criteria were met: i) negative and solvent control show a regular background growth ii) spontaneous reversion rates in the negative and solvent control are in the range of the laboratory's historical control data, iii) positive control substances at least produce a twofold increase compared to the colony count of the corresponding solvent control, and iv) a minimum of five evaluable dose levels were present with at least three dose levels showing no signs of toxic effects, evident as a reduction in the number of revertants below the indication factor of 0.5. A substance was considered mutagenic, if the number of revertants shows a biologically relevant increase exceeding the threshold of twice the colony count of the corresponding solvent control. A dose dependent increase was considered biologically relevant, if the threshold is exceeded at more than one dose level. An increase exceeding the threshold at only one dose level was considered biologically relevant, if it was reproducible in an independent second experiment. A dose dependent increase in the number of revertant colonies below the threshold was regarded as an indication of a mutagenic potential, if it was reproducable in an independent second experiment. However, whenever the colony counts remained within the historical range of negative and solvent controls such an increase was not considered biologically relevant.

2.4. Test chemicals

2,5-Dichlorosulfanilic acid (CAS Registry Number (CASRN) 88-50-6, 98% purity), aniline-2,5-disulfonic acid (98-44-2, 95%), 1,2,4-triaminobenzene dihydrochloride (615-47-4, 96%), 3,3'-dihydroxybenzidine (2373-98-0, 95%), 4-amino-3-methylphenol (2835-99-6, 98%), 1,1-bis(4-aminophenyl)cyclohexane (3282-99-3, 98%), 3,5-dimethyl-1H-pyrazol-4-amine (5272-86-6, 95%), 4,6-diaminoresorcinol dihydrochloride (15791-87-4, 98%), 4-amino-3-hydroxy-N-(2-methoxyphenyl)-2-naphthamide (23342-49-6, 95%), N4-ethyl-N4-(2-hydroxyethyl)-2-methyl-1,4-phenylenediamine sulfate (25646-77-9, 98%), (4-amino-2-methylphenyl)dimethylamine (27746-11-8, 95%), 2-amino-1-naphthol hydrochloride (41772-23-0, 98%), 3-methyl-1-phenyl-1H-pyrazole-4,5-diamine (52943-88-1, 95%), and 4-(4-aminophenyl)thiomorpholine 1,1-dioxide (105297-10-7, 98%) were purchased from abcr GmbH (Karlsruhe, Germany). 2,2′-Dimethylbenzidine (84-67-3, 95%), 2-(4-amino(ethyl)anilino) ethanol (92-65-9, 98%), 3-amino-4-chlorobenzenesulfonic acid (98-36-2, 97%), 2-amino-1,5-naphthalenedisulfonic acid (117-62-4, purity not determined), 4-amino-1,3-benzenedisulfonic acid (137-51-9, 97%), N4,N4-diethyl-2-methyl-1,4-benzendiamine hydrochloride (2051-79-8, 97%), N-(4-Amino-3-methylphenyl)-N-ethylbenzamide (5856-00-8, 95%), 4-(1-(4-amino-3-methylphenyl)cyclohexyl)-2-methylphenylamine (6442-08-6, 95%), were obtained from Sigma-Aldrich Schweiz AG (Buchs SG, Switzerland). 1,2,4,5-Benzenetetraamine tetrahydrochloride (4506-66-5, 97%) was purchased from ChemPur (Karlsruhe, Germany).