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Genotoxic and carcinogenic products arising from reductive transformations of the azo dye, Disperse Yellow 7

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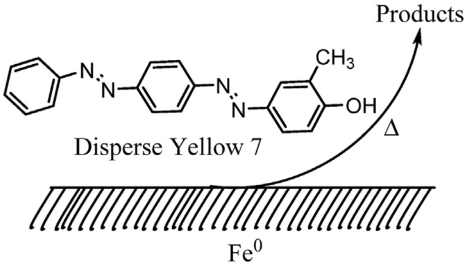
HIGHLIGHTS

- Using LC-QToF-MS, reduction products of Disperse Yellow 7 (a dye) were identified.
- The reduction products we identified are known genotoxins and human carcinogens.
- In the dyeing process, these toxins are formed and likely enter the environment.
- When DY 7 is exposed to aquatic organisms in sediment, the same (geno)toxins arise.

GRAPHICAL ABSTRACT



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ABSTRACT

Selected aromatic azo and benzidine based dyes are priority compounds under the Government of Canada's Chemical Management Plan (CMP) for environmental risk assessments. Organic compounds undergo chemical and biological transformations when they interact with environmental matrices and biotic species; identifying the transformation products is thus a critical component of the risk assessment process.

Here, we used zero valent iron (ZVI) to initiate the reduction of the diazo compound dye **Disperse Yellow 7** (**DY 7**). Using state-of-the-art accurate mass Liquid Chromatography-Quadrupole Time of Flight-Mass Spectroscopy (LC-QToF-MS), four transformation products were conclusively identified, while a fifth product was tentatively ascertained. The conclusively established transformation products included *p*-phenylenediamine (**p-PDA**, a known genotoxin), 4-aminoazobenzene (**4-AAB**, a category 2 carcinogen) and 4-aminobiphenyl (**4-ABP**, a category 1 human carcinogen). **4-ABP** is thought to form *via* a benzidine rearrangement; this is the first report of **DY 7** undergoing a benzidine rearrangement.

Given the importance of reduction processes in the metabolism of organic contaminants by aquatic species, we used LC-MS/MS to analyze sediment samples that had been generated previously upon exposure of Western clawed frogs (*Silurana tropicalis)* to **DY 7** (at exposure levels where cellular stress was observed in *S. tropicalis*). We found **p-PDA**, **4-AAB**, and **4-ABP** were present in all exposures, but not in any of the sediment controls, demonstrating that upon release of **DY 7** to the aquatic environment,

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sediment dwelling organisms will metabolize **DY 7** to generate known (and suspected) human carcinogens, including through a previously unreported *in vivo* benzidine rearrangement to produce **4-ABP**.

1. Introduction

Due to apprehensions regarding their effects on both environmental and human health, synthetic dyestuffs are a significant environmental concern (Reife and Freeman, 1996; Brown and DeVito, 1993; Vaidya and Datye, 1982; Environment Canada and Health Canada, 2012; Pielesz et al., 2002). Extensively used in many fields, synthetic dyes are used in personal care products (*e.g.*, hair colour, deodorant, etc.), food colouring, paper production, leather tanning, as well as textiles (Reife and Freeman, 1996; Vaidya and Datye, 1982). Despite various approaches to treating wastewater effluents (*e.g.*, coagulation, adsorption, chemical decomposition *via* advanced oxidation, and biological treatments (Reife and Freeman, 1996; Forgacs et al., 2004), fully removing these organic pollutants has proven to be quite difficult. Consequently, it is estimated that globally, approximately 10 000 tons of synthetic dyes enter aquatic ecosystems (Forgacs et al., 2004) where very little is known about their ultimate fate and impact, including information regarding the identity of the degradation products that arise from abiotic and/or biological transformations of the parent dye in the environment. As a result, the environmental impact of these transformation processes is difficult to assess. However, regulatory agencies are now working to overcome this issue by identifying possible degradation products arising from dyes (US Environmental Protection Agency, 2010). Under the Government of Canada's Chemical Management Plan (CMP), selected aromatic azo and benzidine based dyes have been targeted as priority compounds for environmental risk assessments; in this manuscript, we present our findings regarding the degradation of one such targeted compound, the azo dye, Disperse Yellow 7 (**DY 7**).

While there is a paucity of information on the global use patterns and environmental occurrence of **DY 7**, according to the US EPA Inventory Update Reporting Program, between 5 and 220 tonnes of **DY 7** were produced in the USA, in each year between 1986 and 2002 (US Environmental Protection Agency, 2002). Recent studies have assessed **DY 7** toxicity to aquatic organisms, including fish (*Pimephales promelas*; Parrott et al., 2015) and frogs (*Silurana tropicalis*; Mathieu-Denoncourt et al., 2014). In both cases, **DY 7** was found to be associated with a toxic response, and in the case of *S. tropicalis*, deformity rates increased at higher **DY 7** exposure. In order to understand the molecular mechanisms of action of **DY 7** in frogs, the authors exposed *S. tropicalis* to **DY 7** at levels associated with toxic responses (albeit sub-lethal) and real-time RT-PCR assays revealed increases in mRNA levels of heat shock protein 70 and heat shock protein 90; both genes are involved in stabilizing proteins and thus protecting them from cellular stress (Mathieu-Denoncourt et al., 2014). At the time of the study, **DY 7** degradation products had yet to be identified, so the authors were unable to begin differentiating between the toxicity/teratogenicity attributable to the parent molecule and that attributable to possible transformation products.

Upon entering aquatic ecosystems, small organic molecules are susceptible to chemical, biological, or environmental transformations (Karickhoff, 1981). Both Maguire (1992) and Weber and Adams (1995) found Disperse Blue 79 (an azo dye) underwent anaerobic reduction in sediments to produce 2-bromo-4,6-dinitroaniline in the water and sediment near the textile mills at Yamaska river in Quebec, Canada (Maguire, 1992; Weber and Adams, 1995). In other cases, various azo dyes have been observed to reductively transform into genotoxic aromatic amines (Brown and DeVito, 1993; Pielesz et al., 2002; Chung, 1983). For example, Pielesz et al. (2002) demonstrated that substituted anilines arose from Sudan III and Disperse Yellow 7 (**DY 7**) while benzidine could arise from Direct Blue 7 and Acid Red 85. Moreover, the dye Direct Blue 14 was shown to form a carcinogenic amine upon exposure to human skin bacteria (Platzek et al., 1999), while other types of dyes (*e.g.*, benzidine dyes) have been shown to undergo reduction in natural waterways, with degradation products that include amines, some of which are known carcinogens (Rindle and Troll, 1975). In textile industries, dyeing processes typically require high temperatures (Perkins, 1991) with temperatures ranging from 120 °C to 230 °C being employed to fix disperse azo dyes (such as Disperse Yellow 7) onto polyester fibers (Perkins, 1991; Aspland, 1992). Since disperse dyes have limited aqueous solubility, particulate (undissolved) dye is often occluded on fiber surfaces upon completion of the dyeing process; this excess, unwanted dye is removed through *reductive clearing*, a process which involves washing the fabric in a sodium dithionite solution at approximately 70 °C (Aspland, 1992). Given that effluents generated by textile mills are subsequently released into the aquatic environment, ascertaining the expected products of disperse dyes such as DY 7 under reducing conditions and elevated temperatures is of direct environmental consequence.

While sodium dithionite (a soluble reducing agent) is used industrially to reductively clear unwanted disperse dyes, its use in the present study would require the use of a quenching agent to terminate the reaction at timed intervals for subsequent analysis. Injecting a pseudo-order excess of dithionite salt and a quenching agent into LC-MS instrumentation would worsen chromatography and increase the susceptibility of the target analytes to matrix effects. Furthermore, it should be noted that sodium dithionite decomposes autocatalytically in water (Lem and Wayman, 1970) to form sodium bisulfate and sodium bisulfite (i.e., decreasing solution pH, which in turn accelerates the degradation of sodium dithionite). Conducting detailed degradation studies in which the compound responsible for the reduction of the target analyte is itself subject to an autocatalytic degradation would result in unnecessarily complex kinetic analyses. By contrast, the reductive decomposition of organic compounds can also be effectively induced using reducing agents such as Zero Valent Iron (ZVI), which, due to its insolubility, would enable the reaction to be quenched by simply filtering the solution to remove Fe^0. Consequently, the unnecessarily complicated kinetic analyses as well as the negative effects on chromatography and sensitivity arising from sodium dithionite usage can be avoided by using ZVI instead.

ZVI has been shown to reduce a variety of organic compounds, including chlorinated alkanes and alkenes (Johnson et al., 1996; Shirin and vanLoon, 2004), polychlorinated dioxins (Kim et al., 2008), explosives (Balakrishnan et al., 2004), and even azo dyes (Weber and Adams, 1995; Bokare et al., 2007; Devi et al., 2009; Nam and Tratnyek, 2000; Cao et al., 1999). While many techniques exist describing the analysis of dyes and their degradation products (Pielesz et al., 2002; Weber and Adams, 1995; Bokare et al., 2007; Devi et al., 2009; Abbott et al., 2009; Rehorek and Plum, 2007;