

Contact Allergy to Textile Dyes – Clinical and Experimental Studies on Disperse Azo Dyes

LAURA MALINAUSKIENE

Vilnius University hospital Santariskiu Clinics, Pulmonology and Allergology Department, Vilnius, Lithuania.

E-mail: laura.malinauskiene@santa.lt

Laura Malinauskiene defended her thesis on 7th December 2012 in Malmö, Sweden. The thesis was supervised by Associate Professor Marlène Isaksson from the Department of Occupational and Environmental Dermatology, Skåne University Hospital Malmö, Lund University, Sweden. The co-supervisors were Associate Professor Erik Zimerson from the Department of Occupational and Environmental Dermatology, Skåne University Hospital Malmö, and Kristina Ryberg, MD, PhD, from Uddevalla Hospital Dermatology Department, Uddevalla, Sweden. The opponent was Professor Tove Agner from Bispebjerg Hospital, Copenhagen University. Associate Professor Liselott Lindh, Malmö, Associate Professor Niels Bendsøe, Lund and Associate Professor Bodil Persson, Lund were members of the assessment committee.

The dyed fibres of the flax plant from Dzudzuana cave in the Caucasus Mountains (Republic of Georgia) are the oldest example of a fabric as they were dated to have existed more than 30 000 years ago. The fact that the fibres were dyed indicates that prehistoric humans were also interested in the exclusiveness of their clothes, not only protection.

In the past, most dyes used for dyeing textile came mostly from nature (e.g., insects or plants). Today most dyes are made from coal tar and petroleum. The chemical structure of synthetic dyes is relatively easy to modify, so many new colours and types of dyes have been synthesised. It is estimated that today some 9,000 colourants with more than 50,000 trade names are used.

Disperse dyes constitute one of the largest dyestuff class, which accounts for 22% of all dyes produced in the world. They have low solubility in water, so the dyes are mixed with a dispersing agent, e.g. lignin sulphonate, naphthalene sulphonate or lignosulphonate to make them soluble in water. Disperse dyes are currently used to dye cellulose acetate, cellulose triacetate, synthetic polyamides, and to a lesser degree, polyacrylonitrile and polypropylene. They are not used to dye natural fibres (wool, silk, cotton). Disperse azo dyes are characterised by the presence of one or more azo groups ($-N=N-$) in the chemical structure. Depending on the fastness of the dyed fabric, these dyes can be removed by rubbing and/or by exposure to water. Besides, disperse dyes are also the most common allergy-causing textile dyes.

We patch-test our dermatitis patients with a mixture of 8 disperse dyes since 1999 in the Department of Occupational and Environmental Dermatology, Skåne University Hospital Malmö. Most patients who have been tested with these 8 dyes reacted to two substances, namely Disperse (D) Orange 1 and D Yellow 3. From previous studies we already knew that commercial D Orange 1 and D Yellow 3 contain more substances than the dye. It is known that some bacteria can



Fig. 1. Laura Malinauskiene defended her PhD thesis in Lund University. Marlène Isaksson (*middle*) has acted as supervisor and Tove Agner (*left*) from Copenhagen University was the opponent. Magnus Bruze (*right*) was the chairman.

cleave azo bond in the dye structure. Recently azoreductases were found to be present in some strains of skin bacteria. If there is a reductive cleavage of azo bond in disperse azo dyes on the surface of the skin or in the skin, aromatic amines could be formed and absorbed through the skin. These metabolites could then induce contact allergy. Before this thesis, it was not known whether the purified dyes, impurities in the commercial dyes or metabolites are the actual allergens for a patient who tests positive on patch testing with D Orange 1 or D Yellow 3. Moreover, it was not known whether these disperse dyes that now are available in commercial test series actually are used in textile dyes today.

A key purpose of this thesis was to evaluate the significance of the contaminants found in commercial dyes D Orange 1 and D

Yellow 3 and their potential metabolites, which can be formed by azoreduction, with regard to contact allergy. A further aim was to investigate the sensitising capacity of D Orange 1 and its metabolites as well as examine their cross-reactivity to D Yellow 3, its metabolites and *p*-phenylenediamine (PPD). A third aim was to investigate whether 8 disperse dyes, most quoted to be allergenic in the medical literature, are still used to colour synthetic fabrics sold in different countries of the world. In addition, we reviewed many published studies dealing with contact allergy to disperse dyes. The thesis was based on 4 scientific papers and one review article.

It is known that some patch tests preparations of disperse dyes contain impurities but their importance for the elicitation of contact allergy has not been established for most dyes. In the first study we examined the significance of these contaminants in the commercial dyes D Orange 1 and D Yellow 3. Ten patients with known contact allergy D Orange 1 and/or D Yellow 3 were patch-tested with dilution series of these dyes, both in the commercial and the purified forms. Nine patients were also tested with a thin layer chromatograms made from the commercial dye. Chromatograms were made by thin layer chromatography (TLC) method on a special plastic film coated with silica gel where separation of different substances present in the commercial form of the dye occurred. Patients were also tested with paper chromatograms made from the water-soluble portions of the respective commercial dyes. The strips were added on the skin as a patch test. Using TLC, we were able to show that the commercial dyes D Orange 1 and D Yellow 3 contained at least 6 impurities each and that these two colours contained at least one contaminant, which was sensitising. These contaminants are not identified yet.

It is known that human skin bacteria can disrupt disperse azo dye to the corresponding aromatic amines *in vitro*. Some of them were found to be allergenic when animal studies (local lymph node assay) have been performed. If the dye molecules detach from the garment, and stick to the skin, these could be broken down by skin bacteria and then penetrate the skin and induce contact allergy. We tested this hypothesis in the second study by patch testing 10 patients with known allergy to D Orange 1 and/or D Yellow 3 with serial dilutions of these two purified dyes, 4-nitroaniline and *p*-aminodiphenylamine in concentrations equimolar to the purified D Orange 1 and also 4-aminoacetanilide and 2-amino-*p*-cresol in concentrations equimolar to the purified D Yellow 3. The observed over-representation of concurrent positive reactions between D Orange 1 and *p*-aminodiphenylamine and between D Yellow 3 and 2-amino-*p*-cresol suggests that these substances can cause sensitisation after being formed on or in the skin. We also suspect cross-reactivity between disperse dyes and these metabolites, since they are exact copies of the ends of the parental compounds. Positive test reactions to D Orange 1 and D Yellow 3 had a tendency to a lower elicitation threshold than for *p*-aminodiphenylamine or 2-amino-*p*-cresol. Thus our observations do not support metabolite theory and results of

threshold values contradict this theory. On the other hand, these observations indicate that simultaneous reactions between D Orange 1 and *p*-aminodiphenylamine and between D Yellow 3 and 2-amino-*p*-cresol are cross-reactions.

We did not know whether the disperse dyes used in commercial patch test series actually are used today to dye synthetic fabrics. Therefore we examined the prevalence of the 8 disperse dyes, mostly described as allergens in the medical literature, in synthetic fabrics purchased at several locations in the world (third study). Textiles from 13 countries in Europe, Asia and the United States were analysed using TLC. When there were corresponding spots comparing extract made from textile with the reference dye, high pressure liquid chromatography was performed. Among the 121 analysed garments we found 4 of the dyes in 3 different garments. It was a pair of light brown tights manufactured and sold in Italy and containing D Yellow 3, D Blue 124 and D Blue 106, and a bra and panty set from India where we found D Orange 1. So disperse dyes today we use for patch testing are not widely used for dyeing clothes anymore but one can still find them in some garments, and even in those that are manufactured in Europe.

In the fourth study, we reviewed the published studies dealing with contact allergy to disperse dyes used for dyeing textiles. We searched articles on PubMed from the period 1990–2012. We found 54 studies where 26 disperse dyes were described as allergens. Positive patch test reaction prevalence rates at least to 3 dyes (D Blue 106, D Blue 124 and D Orange 3) were over 1% in screening dermatitis patients. We found no data for D Blue 26 and 102, D Orange 37 and D Yellow 49, all of which are listed as allergens by the EU Commission. In the fifth study, we investigated the sensitizing capacity of D Orange 1 and its two metabolites, *p*-aminodiphenylamine and 4-nitroaniline and cross-reactivity between them and also with D Yellow 3, its two metabolites, 4-aminoacetanilide and 2-amino-*p*-cresol, and one potential cross-reactant, PPD, by performing the guinea pig maximization test. We found that D Orange 1 and *p*-aminodiphenylamine are strong allergens and they cross-react with each other. Neither PPD, 4-nitroaniline, 4-aminoacetanilide, 2-amino-*p*-cresol nor D Yellow 3 showed cross-reactivity to D Orange 1 or *p*-aminodiphenylamine. The other potential metabolite of D Orange 1, 4-nitroaniline, was not an allergen in the guinea pig test.

With the results presented in the thesis, we concluded that:

- It would be useful to examine which disperse dyes are used today to dye textiles and then modify patch test series. When we examined textiles from different parts of the world, we saw similar colour pattern among many extracts, especially in the orange, blue, red and yellow spectra.
- The purity of the dyes used today for patch testing should be investigated and the relevant pollutants identified in order to be able to define their sensitizing capacity.
- Late readings of patch test reactions of these substances should be performed because some allergic reactions comes after day 3 or 4.

- One way to find new markers for textile dye contact allergy would be to examine workers in textile dyeing industry with clinical examination and patch testing with working material.

It would also be of value if the substances present as impurities in the commercial dyes that our patients reacted on patch testing would be identified.

Since azoreduction can possibly take place on or in the skin, patch testing patients with known allergy to D Orange 1 and/or D Yellow 3 with these substances in the dilution series in

both normal and disinfected skin would allow to get more knowledge about the role of the skin bacteria in allergy development.

To determine whether the D Orange 1 itself or its metabolites are the primary allergen these substances should be tested equimolar and in dilution series in the elicitation phase in the guinea pig maximisation test. Also further insight into the absorption and metabolism of disperse dyes in the skin could lead to more knowledge about contact allergy to textile dyes.