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An animal model assessment of common dye-induced allergic contact dermatitis

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Synopsis

Dyes are a category of substances capable of inducing allergic contact dermatitis found in a variety of foods, drugs, textiles, cosmetics, and hair dyes. This study tested 33 dyes in guinea pigs using a modified Buehler and Klecak method for open epicutaneous testing. The dyes were tested at an induction concentration of 10% and challenge concentrations of 10.0%, 5.0%, and 2.5%. Nine of the 33 dyes tested produced positive allergic reactions in the guinea pig model (2-amino-4-nitrophenol, 2-amino-5-nitrophenol, acid yellow 23, acid orange 3, basic black 3, basic orange 1, disperse orange 3, solvent black 27, and solvent black 34). When eight of the nine positive dyes were retested using a 1% induction concentration, five dyes produced allergic contact dermatitis at a 1% challenge concentration (2-amino-4-nitrophenol, 2-amino-5-nitrophenol, acid yellow 23, disperse orange 3, and solvent black 34), two at a 0.5% challenge concentration (2-amino-5-nitrophenol and solvent black 34), and one at a 0.25% challenge concentration (2-amino-5-nitrophenol). DNCB at a 0.5% induction/challenge concentration was used as a positive control. With the exception of disperse orange 3 and acid yellow 23, the seven additional dyes that elicited positive allergic reactions in the guinea pig model have not been previously reported.

INTRODUCTION

Dyes are a chemical category capable of causing allergic contact dermatitis found in foods, drugs, textiles, cosmetics, and hair dyes. The most common dye sensitizer is paraphenylenediamine (PPD), which was given a significance prevalence index number (SPIN) ranging from 3 to 10 between the years 1984 and 1996 by the North American Contact Dermatitis Group (1). Another well known allergenic dye is acid yellow 23, also known as tartrazine or FD&C yellow No. 5, widely reported to produce food and drug allergies with cross sensitization potential to aspirin (2–5). Other allergenic dyes reported in the literature include disperse blue 106, disperse blue 124, disperse blue 153, disperse brown 1, disperse orange 3, disperse orange 13, disperse red 1, and disperse yellow 3 (6–9). Some of these dyes have also been shown to cross react with PPD

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(10–12), although PPD sensitivity is not always a reliable indicator for general dye sensitivity. With the exception of disperse orange 3 and acid yellow 23, the dyes that elicited positive allergic reactions in this guinea pig model research have not been previously reported in the literature.

METHOD

All test materials were evaluated using a modification of the Buehler and the Klecak method for open epicutaneous testing (OET) for sensitization in a guinea pig model (13–16). Our methodology utilized induction and challenge periods. For the induction phase, the left flanks of ten albino guinea pigs were shaved and the dye test material applied three times weekly (Monday, Wednesday, Friday) for three consecutive weeks. Each animal received 0.1 ml of the dye test material over a 1.8-cm circular area. Following the induction period, the guinea pigs entered the challenge phase. The challenge phase began after a two-week rest period when the right flank of each guinea pig was shaved and exposed to three different dye test material concentrations (100%, 50%, and 25% of the induction concentration). Twenty-four hours after the last induction and challenge application, the animals were depilated to clearly observe dermal reactions.

All test sites were graded for erythema and edema 24 and 48 hours post-application using a four-point ordinal scale (0 = no reaction, 1 = slight reaction, 2 = moderate reaction, 3 = severe reaction). A positive reaction was defined as an erythema/edema value during the challenge phase of at least one skin grade higher than during the last induction phase. For example, a challenge grade of 1 would be considered positive if a 0 was noted for the animal 24 hours after the last induction application. In addition to the dye test agents evaluated, a positive control of 0.5% 2,4-dinitrochlorobenzene (DNCB) in ethanol was included for both the induction and challenge phases.

RESULTS

Table I outlines the results obtained for the 33 dye ingredients tested in the guinea pig model at an induction concentration of 10% in propylene glycol (PG) and challenge concentrations of 10.0%, 5.0%, and 2.5% in PG. Of the 33 dyes tested, nine dyes demonstrated positive allergic reactions at the 10.0% challenge concentration (2-amino-4-nitrophenol, 2-amino-5-nitrophenol, acid yellow 23, acid orange 3, basic black 3, basic orange 1, disperse orange 3, solvent black 27, and solvent black 34), six at the 5.0% concentration (2-amino-4-nitrophenol, 2-amino-5-nitrophenol, acid orange 3, basic black 3, basic orange 1, and disperse orange 3), and four at the 2.5% concentration (2-amino-4-nitrophenol, acid orange 3, basic black 3, and basic orange 1). The positive control, DNCB at the 0.5% induction/challenge concentration, elicited positive responses in all animals tested. When eight of the nine positive dye test compounds were retested using an induction concentration of 1% in PG, five dyes demonstrated positive reactions at the 1% challenge concentration (2-amino-4-nitrophenol, 2-amino-5-nitrophenol, acid yellow 23, disperse orange 3, and solvent black 34), two at the 0.5% challenge concentrations (2-amino-5-nitrophenol and solvent black 34), and one at the 0.25% challenge concentration (2-amino-5-nitrophenol). These results are summarized

Table I
Dyes Tested

Colour index name (other names)[1]	*Colour index* no. or chemical abstract no.	Chemical class	Induction conc. in propylene glycol	Challenge conc. in propylene glycol[2]		
				10.00%	5.00%	2.50%
2-Amino-4-nitrophenol	99-57-0	Nitroaminophenol	10.00%	+ (80%)	+ (70%)	+ (40%)
2-Amino-5-nitrophenol	121-88-0	Nitroaminophenol	10.00%	+ (30%)	+ (10%)	Negative
Acid black 2	50420	Azine	10.00%	Negative	Negative	Negative
Acid black 24	26370	Azo	10.00%	Negative	Negative	Negative
Acid blue 1 (Food blue 3)	42045	Triphenylmethane	10.00%	Negative	Negative	Negative
Acid blue 45	63010	Anthraquinone	10.00%	Negative	Negative	Negative
Acid brown 359	Not assigned	Monoazo	10.00%	Negative	Negative	Negative
Acid yellow 23 (Tartrazine/yellow 5)	19140	Monoazo	10.00%	+ (20%)	Negative	Negative
Acid orange 3	10385	Nitro	10.00%	+ (80%)	+ (60%)	+ (30%)
Acid red 50	45220	Xanthene	10.00%	Negative	Negative	Negative
Basic black 3	11825	Monoazo	10.00%	+ (100%)	+ (100%)	+ (50%)
Basic orange 1	11320	Monoazo	10.00%	+ (100%)	+ (80%)	+ (70%)
Basic red 2	50240	Azine	10.00%	Negative	Negative	Negative
Basic violet 1 (Gentian violet)	42535	Triarylmethane	10.00%	Negative	Negative	Negative
Basic violet 2	42520	Triarylmethane	10.00%	Negative	Negative	Negative
Basic violet 3 (Gentian violet)	42555	Triarylmethane	10.00%	Negative	Negative	Negative
Basic violet 13	42536	Triarylmethane	10.00%	Negative	Negative	Negative
Disperse black 9	12222-69-4	Azo	10.00%	Negative	Negative	Negative
Disperse blue 1 (Solvent blue 18)	64500	Anthraquinone	10.00%	Negative	Negative	Negative
Disperse orange 3 (Solvent orange 9)	11005	Monoazo	10.00%	+ (50%)	+ (30%)	Negative
Disperse red 55	Not assigned	Anthraquinone	10.00%	Negative	Negative	Negative
Disperse violet 4 (Solvent violet 12)	61105	Anthraquinone	10.00%	Negative	Negative	Negative
Disperse violet 11	Not assigned	Anthraquinone	10.00%	Negative	Negative	Negative
Disperse yellow 49	Not assigned	Methine	10.00%	Negative	Negative	Negative
Disperse yellow 232	55165	Azo	10.00%	Negative	Negative	Negative
HC red 3	1/4/2871	Nitrophenylenediamine	10.00%	Negative	Negative	Negative
HC yellow 4	59820-43-8	Nitroaminophenol	10.00%	Negative	Negative	Negative
HC yellow 5	56932-44-6	Nitrophenylenediamine	10.00%	Negative	Negative	Negative
Solvent black 5	50415	Azine	10.00%	Negative	Negative	Negative
Solvent black 27	Not assigned	Not assigned	10.00%	+ (70%)	Negative	Negative
Solvent black 34	12195	Monoazo	10.00%	+ (100%)	Negative	Negative
Solvent blue 6	44040:1	Triarylmethane	10.00%	Negative	Negative	Negative
Solvent red 122	Not assigned	Azo	10.00%	Negative	Negative	Negative

[1] Information pertaining to *Colour Index* nos. and chemical class obtained from *Colour Index* (17).
[2] Results expressed as negative or positive (+) with the percent of animals in the group demonstrating an allergic reaction in parentheses.

Table II
Dose Response of Dyes Eliciting Positive Reactions at the 10% Level

Colour index name (other names)[1]	*Colour index* no. or chemical abstract no.	Chemical class	Induction conc. in propylene glycol	Challenge conc. in propylene glycol[2]		
				1.00%	0.50%	0.25%
2-Amino-4-nitrophenol	99-57-0	Nitroaminophenol	1.00%	+ (30%)	Negative	Negative
2-Amino-5-nitrophenol	121-88-0	Nitroaminophenol	1.00%	+ (40%)	+ (20%)	+ (10%)
Acid yellow 23 (Tartrazine/yellow 5)	19140	Monoazo	1.00%	+ (10%)	Negative	Negative
Acid orange 3	10385	Nitro	1.00%	Negative	Negative	Negative
Basic black 3	11825	Monoazo	Not tested		Not tested	
Basic orange 1	11005	Monoazo	1.00%	Negative	Negative	Negative
Disperse orange 3 (Solvent orange 9)	11005	Monoazo	1.00%	+ (10%)	Negative	Negative
Solvent black 27	Not assigned	Not assigned	1.00%	Negative	Negative	Negative
Solvent black 34	12195	Monoazo	1.00%	+ (20%)	+ (20%)	Negative

[1] Information pertaining to *Colour Index* nos. and chemical class obtained from *Colour Index* (17).
[2] Results expressed as negative or positive (+) with the percent of animals in the group demonstrating an allergic reaction in parentheses.

in Table II. The positive DNCB control at the 0.5% induction/challenge concentration elicited positive responses in all animals tested.

DISCUSSION

Dye allergies can be a challenging problem for the sensitized consumer. To avoid offending substances, labels are difficult to read without knowledge of dye chemistry. Furthermore, it is very difficult for the dermatologist to treat and diagnose allergies to commonly used dyes. The dyes tested in this research can be found in clothing fabrics, such as cotton, wool, nylon, silk, acrylic, and acetate; animal skins used for clothing, such as furs, sheepskin, leather, and suede; bast fibers used in paper, rope, and clothing (juts, flax, sunn, hemp, and ramie); inks (general printing inks, pen inks, marking pens, stamp pads); paper (colored paper and carbon paper); solvents used for painting/staining/repairing furniture (lacquers, varnishes, wood stains, and resins); colored plastics (polystyrene, vinyl, and pvc); shoe polishes; photographic filters; colored metals (anodized aluminum); and even biological stains used in various types of laboratories. From the standpoint of the cosmetic chemist, the most common use of these dyes is in soaps and hair dyes.

There are some reports in the literature of dye allergies that were not confirmed by this research. For example, three dyes studied did not elicit a positive allergic reaction in guinea pigs (disperse blue 1, disperse red 55, and disperse yellow 49), but were reported in *Fisher's Contact Dermatitis* as "known to be potentially allergenic" (5). One possible explanation for this phenomenon, which is implied in Fisher's text, is that the "frequency of cross-reactivity between Azo dyes and PPD could be explained by Mayer's hypothesis that they shared common transformation to quinonediimine by cells of the skin" (5). It is possible that this transformation did not occur in the guinea pig skin, accounting for these findings.

Some of the dyes studied in this research are known by different names depending upon their intended use. For example, acid yellow 23, which is considered a non-certified dye, is known as FD&C yellow no. 5 or yellow 5 when the heavy metals are removed. This dye was found to be allergenic in our guinea pig model and may be labeled under several different names.

This research has generated a list of commonly used dyes that may demonstrate an increased risk of allergic contact dermatitis in humans, based on guinea pig findings. This list may provide a reference for the cosmetic chemist, who wishes to develop products that could be considered hypoallergenic, by avoiding known allergenic dyes. The hypoallergenic label, which means reduced allergy, not non-allergic, is especially important in hair dyes. Dye-sensitive consumers have the greatest problem with hair dyes because the ammonia and/or hydrogen peroxide present in the hair dye enhances penetration of the allergen into the skin, increasing exposure. The allergenic dye may also be left in contact with the skin for 20 to 40 minutes, allowing sufficient time for an immune response. Dye allergies have a dramatic presentation, with total facial swelling so severe that the eyes cannot be opened. Avoiding the dyes identified in this research as allergenic may be important in the development of products bearing the hypoallergenic claim.

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