Journal of the American College of Toxicology
15(4):311–319, Lippincott–Raven Publishers, Philadelphia

Final Report on the Safety Assessment of Disperse Yellow 3[1]

Abstract: Disperse Yellow 3 is a monoazo dye for which the exclusive cosmetic use has been in hair dyes. It is not, however, currently reported to be used. The toxicity of 87.6% pure Disperse Yellow 3 dye in mice and rats was found to include death; dose-related pituitary, thyroid, spleen, and kidney lesions; and carcinogenesis in male rats and female mice. Mutagenicity was observed in several systems, but the impact of activation was inconsistent. Clinical data suggest that some individuals exhibiting contact allergy to dyes in general may be sensitive to Disperse Yellow 3. Because there is an absence of data on the actual dermal absorption of this ingredient, the conclusion was reached that the available data are insufficient to support its safety. If the ingredient were found to be significantly absorbed, the conclusion would be that the ingredient is unsafe based on its carcinogenic potential. If the ingredient were found not to be absorbed, then data on impurities and on ocular toxicity would be needed to reach a more definitive conclusion regarding safety. **Key Words:** Disperse Yellow 3—Monoazo dye—Hair dye—Carcinogenesis—Allergy—Dermal absorption.

CHEMISTRY

Definition and Structure

Disperse Yellow 3 (CAS no. 2832-40-8) is *not* currently listed as a cosmetic ingredient. It is a monoazo dye that has the structure shown in Fig. 1 (International Agency for Research on Cancer [IARC], 1990). Synonyms for Disperse Yellow 3 include 4-acetamido-2′-hydroxy-5′-methylazobenzene, CI solvent yellow 77, CI solvent yellow 92, CI solvent yellow 99, 4′-[(2-hydroxy-5-methylphenyl)azo]acetanilide, and 4′-(6-hydroxymetatolylazo)acetanilide (IARC, 1990).

Physical and Chemical Properties

Disperse Yellow 3 is a solid that decomposes at 192–195°C and is soluble in acetone, ethanol, benzene, and in water at 1.5–6.1 mg/L at 60°C (IARC, 1990).

Methods of Manufacture

Fischer and Muller initially produced Disperse Yellow 3 in 1926 by coupling diazotized 4-acetamidoaniline with para-cresol (IARC, 1990). The method used for commercial production is not known.

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[1] Reviewed by the Cosmetic Ingredient Review Expert Panel.

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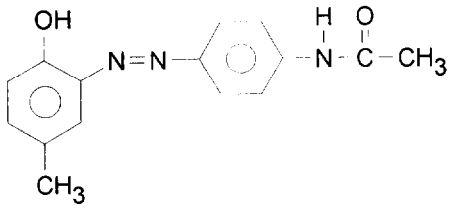

FIG. 1. Chemical formula for Disperse Yellow 3 (IARC, 1990).

USE

Cosmetic

Data submitted to the Food and Drug Administration (FDA) in 1992 by cosmetic firms participating in the voluntary cosmetic registration program indicated that Disperse Yellow 3 was used in nine formulations of oxidative hair dyes and colors (FDA, 1992). It was not listed as being used in the 1993 FDA compilation (FDA, 1993).

Hair-coloring formulations are applied to or may come in contact with hair, skin (particularly the scalp), eyes, and nails. Individuals who dye their hair may use such formulations as often as once a week. Hairdressers may come in contact with such products several times a day. Permanent hair dyes contain couplers and an oxidant in addition to the primary intermediate (the actual dye). Users may be exposed to reactive intermediates as well as to unreacted dyes (Corbett and Menkart, 1973). Any oxidative or permanent hair dyes containing Disperse Yellow 3, as "coal tar" hair dye products, would be exempt from the principal adulteration provision and from the color additive provision in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a caution statement and "patch test" instructions for determining whether the product causes skin irritation. In order to be exempt, the following caution statement must be displayed on all coal tar hair dye products:

> **Caution**—this product contains ingredients that may cause skin irritation on certain individuals, and a preliminary test according to accompanying directions should be made. This product must not be used for dyeing the eyelashes or eyebrows; to do so may cause blindness.

Patch test instructions call for a 24-h patch on the skin of the user with the intermediates and hydrogen peroxide mixed in the same manner as in use. This test is to be performed before each and every application of the hair dye (Corbett and Menkart, 1973).

At its February 11, 1992, meeting, the Cosmetic Ingredient Review (CIR) Expert Panel issued the following policy statement on coal tar hair dye product labeling:

> Since the recommendation on the industry's adopted labeling establishes a procedure for individual user safety testing, it is most important that the recommended procedure be consistent with current medical practice. There is a general consensus among dermatologists that screening of patients for sensitization (allergic contact dermatitis) should be conducted by the procedures used by the North American Contact Dermatitis Group and the International Contact Dermatitis Group (NACDG, 1980; Eiermann et al.,

1982; Adams et al., 1985). Basically, these procedures state that the test material should be applied at an acceptable concentration to the patient, covered with an appropriate occlusive patch, and evaluated for sensitization at 48 and 72 hours after application. The CIR Expert Panel has cited the results of studies conducted by both the North American Contact Dermatitis Group and the International Contact Dermatitis Group in its safety evaluation reports on cosmetic ingredients (Elder, 1985).

During the August 26–27, 1991 public meeting of the CIR Expert Panel, all members agreed that the cosmetics industry should change its recommendation for the evaluation of the open patch test from 24 hours to 48 hours after application of the test material.

The industry was advised of this recommendation and asked to provide any compelling reasons why this recommendation should not be made by the Expert Panel and adopted by the cosmetics industry. No opposition to this recommendation was received. At the February 11, 1992 public meeting of the CIR Expert Panel, this policy statement was adopted.

International

No information is available on the use of this ingredient in cosmetics in other countries.

Noncosmetic

Disperse Yellow 3 is used to color nylon, polyvinyl chloride and acrylic fibers, wools and furs, cellulose acetate, polystyrene, and other thermoplastics. These materials are then contained in such finished products as clothing, hosiery, and carpeting (Society of Dyers and Colourists, 1971).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, Excretion

No specific information is available. It is hypothesized that in mammals the route of metabolism is probably reduction of the azo linkage to produce corresponding aromatic amines (National Toxicology Program [NTP], 1982; Oak Ridge National Laboratory, 1982).

ANIMAL TOXICOLOGY

Acute Oral

Groups of five male and five female B6C3F_1 mice and F344 rats were maintained on feed containing 6,000, 12,500, 25,000, 50,000, or 100,000 ppm Disperse Yellow 3 for 24 h, followed by 14 days of regular feed. All the animals were killed on day 15. No deaths occurred, and no overt signs of toxicity were observed (NTP, 1982).

Short-term Oral

Groups of five male and five female B6C3F$_1$ mice and F344 rats were maintained on feed containing 0, 6,000, 12,500, 25,000, 50,000 or 100,000 ppm Disperse Yellow 3 for 2 weeks. All surviving animals were killed on day 15. Compared with controls, weight gain was decreased by ≥15% in all dosed rats and by at least 25% in all dosed mice, except for males receiving 6,000 ppm and females receiving either 12,500 or 50,000 ppm. Of the animals receiving 100,000 ppm, all rats, four male mice, and two female mice died before day 15. Of those receiving 50,000 ppm, one male rat and one female rat died. Rats receiving 100,000 ppm had depleted fat deposits around the kidneys, adrenal glands, and heart. Their spleen, kidneys, and liver were uniformly dark red to black. The organs of rats fed 50,000 ppm had similar characteristics, though to a milder degree. Mice fed 100,000 ppm had similar changes, though they were described as less severe than those found in rats fed the same dosage (NTP, 1982).

Subchronic Oral

Groups of 10 male and 10 female B6C3F$_1$ mice and F344 rats were maintained on feed containing 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm Disperse Yellow 3 for 91 days. The animals were weighed weekly. Necropsy was performed at the end of the dosing period. One male rat receiving 1,250 ppm and one female receiving 20,000 ppm died during the dosing period. Weight gain was decreased by at least 18% in rats that received either 10,000 or 20,000 ppm Disperse Yellow 3. Lesions were observed in rats dosed with ≥5,000 ppm. These findings are summarized in Table 1. The severity of the lesions were dose related. No lesions were observed in control rats or those that received the 1,250- or 2,500-ppm diets. None of the mice died during the dosing period. Mean weight gain was decreased by at least 10% in male mice receiving ≥5,000 ppm and in female mice receiving ≥10,000 ppm. Hemosiderosis of the renal tubular epithelium was observed in all mice that received ≥10,000 ppm Disperse Yellow 3; hemosiderosis of the spleen was observed in all mice that received ≥5,000 ppm; and cytoplasmic swelling of the centrilobular hepatocytes was seen in six males and six females of the 10,000-ppm group and in all males and eight females of the 20,000-ppm group (NTP, 1982).

Chronic Oral

Groups of 100 (50 of each sex) B6C3F$_1$ mice and F344 rats were maintained for 103 weeks on feed containing Disperse Yellow 3. (The test material contained 87.6% dye.) Rats received either 5,000 or 10,000 ppm Disperse Yellow 3 in the feed, while mice received either 2,500 or 5,000 ppm of the test material. The doses had been determined from results of 13-week subchronic feeding studies conducted earlier (see Animal Toxicology, Subchronic Oral). Groups of 50 animals of each sex and species received untreated feed and served as controls. All animals were observed for at least 105 weeks. Feed consumption was comparable between dosed and control groups. Dosed animals of both species, but especially rats, had

TABLE 1. *Histopathologic findings in rats fed Disperse Yellow 3 in diet for 91 days*[a]

Histopathologic findings	Dose (ppm)					
	5,000		10,000		20,000	
	M	F	M	F	M	F
Pituitary, pars distalis vacuolar degeneration	0/10	0/10	0/10	0/10	10/10	6/6
Thyroid, capsule, fibrous thickening	0/10	0/10	10/10	0/10	10/10	9/9
Thyroid, follicular hyperplasia, mild	0/10	0/10	10/10	10/10	0/10	0/9
Thyroid, follicular hyperplasia, moderate	0/10	0/10	0/10	0/10	10/10	9/9
Thyroid, adenomatous hyperplasia	0/10	0/10	0/10	0/10	0/10	1/9
Thyroid, adenoma	0/10	0/10	0/10	0/10	3/10	1/9
Thyroid, capsule, inflammatory infiltrate, nonsuppurative, mild	0/10	0/10	0/10	0/10	1/10	0/9
Spleen, hemosiderosis, mild	4/10	8/10	10/10	10/10	0/10	0/9
Spleen, hemosiderosis, moderate	0/10	0/10	0/10	0/10	10/10	9/9
Spleen, lymphocytic depletion, mild	0/10	0/10	0/10	0/10	10/10	8/9
Kidney, cortical tubules, pigment deposition, mild	0/10	0/10	10/10	10/10	0/10	0/9
Kidney, cortical tubules, pigment deposition, moderate	0/10	0/10	0/10	0/10	10/10	9/9

M, male; F, female.
[a] From the NTP (1982).

depressed body weight gains versus controls. However, treated rats survived significantly longer than did control rats (the survival of control rats was consistent with that of three previous groups of the same strain used in the laboratory).

In male rats, 31 of the control group, 45 of the low-dose group, and 39 of the high-dose group survived to the end of the study. In female rats, 33 of the control group, 40 of the low-dose group, and 46 of the high-dose group survived. Compared with controls, treated male rats had a lower incidence of malignant mesothelioma and C-cell carcinoma of the thyroid gland, and dosed females had a decreased incidence of pituitary adenoma and endometrial stromal polyps. Both male and female dosed rats had significantly lower incidences of lymphocytic leukemia and lymphoma than did control rats. The researchers did not attribute the longer survival in the treated rats to a lower incidence of any single neoplasm (NTP, 1982). More details are available under Carcinogenicity in this report.

MUTAGENICITY

Frog (*Rana clamitans*) larvae (number not specified) were injected once daily with 0.4 ml of Disperse Yellow 3 (prepared in saline at 4 mg/ml) for 7 days. Controls were injected with saline. After the sixth injection, a portion of the tail was removed, and the tail was allowed to regenerate for 5–7 days. Permanent squash slides of regenerating cells were analyzed for chromosomal aberrations. Aberrations were noted in 19 of 31 cells counted. These aberrations consisted of four dicentrics, seven breaks, and six gaps; two aberrations were classified as "other." In the same study, the tails were removed from another set of larvae. These larvae were not injected, but instead were placed in solutions of 800 ml of aged tap water, to which 0.8 ml of the Disperse Yellow 3 solution (4 mg/ml) was

added. Controls were placed in solutions of aged tap water alone. Of the 49 cells observed from the regenerating tadpoles placed in dye suspension, seven cells had gap aberrations. The 70 cells analyzed from both control groups combined had no abnormal chromosomes. In both treatments, Disperse Yellow 3 produced chromosome aberrations, while none were seen in controls (Gray et al., 1979).

Disperse Yellow 3 produced forward mutations at the thymidine kinase locus (tk) in L5178Y clone 3.7.2C mouse lymphoma cells in the presence of 5-trifluorothymidine (McGregor et al., 1988). Cultures of 6×10^6 cells of tk^+/tk^--3.7.2C heterozygote of L5178Y mouse lymphoma cells were exposed to Disperse Yellow 3 at concentrations between 5 and 40 μg/ml in dimethyl sulfoxide. Two cultures at each concentration were maintained in the presence of S9 (postmitochondrial supernatant fractions of liver homogenates) and two in the absence of S9. There were four cultures of the vehicle control and two of the positive control. Cells were sedimented, resuspended, and incubated for a 2-day expression period. Aliquots were removed and plated for cloning and mutation counts. Between two and four plates were counted for each culture. Neither a mutagenic nor a toxic response was observed at any tested concentration of Disperse Yellow 3 without S9 mixture. The relative total growth (RTG, or growth in dosed culture/growth in control culture) remained at ≥76%. In cultures with the S9 mixture, two plates had changes of a toxic response at 10 μg/ml, the lowest observed effective dose. At this dose the RTG was 39%. Furthermore, a significant ($p < 5\%$) average mutant factor of 80 (average mutant colonies per 10^6 clonable cells) was noted at this dose.

In a sister chromatid exchange (SCE) assay using Chinese hamster ovary cells, Disperse Yellow 3 increased the frequency of SCE compared with controls and cell cycle delay at 15 μg/ml without S9 activation. In a second trial, a significant increase in SCE occurred in cells exposed to 5 and 15 μg/ml, but no positive response was detected at 10 μg/ml. (An increase of ≥20% in SCE per chromosome over the solvent control was considered significant.) With S9 activation, however, no significant response was detected for a dose range between 150 and 1,500 μg/ml (Ivett et al., 1989). In this assay, Disperse Yellow 3 was mutagenic with metabolic activation, but not without it.

CARCINOGENICITY

Groups of 50 4-week old male and female F344 rats and $B6C3F_1$ mice were used in a 2-year feeding carcinogenesis bioassay (NTP, 1982). The study design is detailed herein in the Chronic Oral section under Animal Toxicology. Briefly, rats were maintained on feed containing either 5,000 or 10,000 ppm Disperse Yellow 3 for 103 weeks. Mice were dosed with either 2,500, or 5,000 ppm. Treated male rats had a significantly higher incidence of neoplastic nodules of the liver: one of 49 controls (one control animal was not evaluated), 15 of 50 in the low-dose group, 10 of 50 in the high-dose group. Neoplastic nodules or hepatocellular carcinoma developed in two of 49 control males, in 15 of 50 males in the low-dose group, and in 11 of 50 high-dose-group males. Gastric neoplasms were more prevalent in

treated male rats: 0 of 49 controls, four of 50 in the low-dose group, one of 50 in the high-dose group. While the increased incidence was not statistically significant within the study, the historical incidence of gastric neoplasms in controls was reported to be 10/2,960 (0.3%). Thus, the increase in gastric neoplasms was attributed to administration of Disperse Yellow 3. No significant increase in the incidence of neoplasms was noted in female rats.

In mice, low-dose males had a lower incidence of hepatocellular adenomas than did high-dose or control males. Low-dose female mice also had lower incidences of alveolar/bronchiolar adenoma and/or carcinoma than did high-dose or control females. No explanation was offered for this negative trend. In female mice, hepatocellular adenoma was significantly increased in dosed animals: zero controls, six of the low-dose group, 12 of the high-dose group. Similarly, the incidence of hepatocellular adenoma or carcinoma were higher: two controls versus 10 low-dose and 17 high-dose female mice. No significant increase in the incidence of neoplasms was noted in male mice. Disperse Yellow 3 was concluded to be a carcinogen in male F344 rats and female B6C3F_1 mice (NTP, 1982).

CLINICAL ASSESSMENT OF SAFETY

Dermal Sensitization

Five persons allergic to Disperse Yellow 3 were exposed to purified dyes. Patch tests were performed using a 2% concentration in petrolatum. The positive reactions that resulted confirmed that the dye, identified as 4'-acetamido-2-hydroxy-4-methylazobenzene (an isomer of Disperse Yellow 3), and not impurities, was the sensitizer (Foussereau et al., 1972). In another study, epicutaneous testing was performed on 12 eczema patients with suspected contact allergy to textile dyes. Disperse Yellow 3 was tested at a concentration of 1% (in petroleum). Five of the 12 patients had positive reactions (Kousa and Soini, 1980).

Epidemiology

Between 35% and 45% of American women dye their hair, often at monthly intervals over a period of years (Cosmetic, Toiletry, and Fragrance Association, 1993). This estimate is drawn from market research data on hair dye product use, generally from females aged 15 to 60. A number of epidemiologic studies have investigated the association between cancer and occupation as a hairdresser or barber and between cancer and personal use of hair dyes. The World Health Organization's International Agency for Research on Cancer (IARC) empaneled a Working Group on the Evaluation of Carcinogenic Risks to Humans to review all available data on these issues. The Working Group met October 6–13, 1992, in Lyon, France (IARC, 1993). The charge to the IARC Working Group was to ascertain that all appropriate data had been collected and were being reviewed, to evaluate the results of the epidemiological and experimental studies and prepare accurate summaries of the data, and to make an overall evaluation of the carcinogenicity of the exposure to humans.

The IARC Working Group's conclusions were: "There is *inadequate evidence* that personal use of hair colourants entails exposures that are carcinogenic."

Hence: "Personal use of hair colourants *cannot be evaluated as to its carcinogenicity (Group 3).*" The IARC Working Group also concluded: "There is *limited evidence* that occupation as a hairdresser or barber entails exposures that are carcinogenic." Hence: "Occupation as a hairdresser or barber entails exposures that *are probably carcinogenic (Group 2A)*" (IARC, 1993). The Expert Panel concluded that the relevance of the occupational data and conclusion to individuals using hair dyes is unclear.

SUMMARY

Disperse Yellow 3 is a monoazo dye used exclusively in hair dyes. As of 1993, there were no uses of this compound reported to the FDA. In an NTP single-dose study conducted using 87.6% pure dye, no evidence of toxicity was observed in mice and rats maintained on feed containing ≤100,000 ppm of Disperse Yellow 3. In the short-term feed study, 10 of 10 rats and six of 10 mice fed 100,000 ppm died. In the subchronic study, one of 10 rats fed 1,250 ppm and one of 10 rats fed 20,000 ppm died. Dose-related lesions of the pituitary and thyroid glands, spleen, and kidneys were found. In the chronic study, Disperse Yellow 3 was concluded to be carcinogenic in male rats and female mice. Disperse Yellow 3 did induce chromosomal aberrations when injected into tail-regenerating frog larvae and, to a lesser extent, in larvae placed in dye suspensions. A toxic response and an induction of forward mutations was observed in mouse lymphoma cells exposed to a minimum of 10 μg/ml of Disperse Yellow 3 with S9 mixture. Without S9, neither a mutagenic nor toxic response was observed. Disperse Yellow 3 induced SCE in Chinese hamster ovary cells without S9 at 15 μg/ml. With S9 activation no response was detected. A 2% concentration of purified dye induced allergic reactions in five of five people with known allergies to Disperse Yellow 3. In another study of 12 people with suspected contact allergy to dyes, five had positive patch-test reactions to Disperse Yellow 3 at a concentration of 1%.

DISCUSSION

Section 1, paragraph (p) of the CIR Procedures states that a "lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the Procedures, the Expert Panel informed the public of its decision that the data on Disperse Yellow 3 were not sufficient for determination of whether the ingredient, under relevant conditions of use, was either safe or unsafe. The Panel stated that dermal absorption data are needed to assess the safety of Disperse Yellow 3. This request was made because Disperse Yellow 3 was found to have carcinogenic potential. The Panel wished to determine the extent of absorption with the proviso that if a significant amount was absorbed, Disperse Yellow 3 would be found unsafe. In the absence of significant absorption, additional data concerning impurities and ocular toxicity would be required to complete the safety assessment. No comments were received during the 90-day public comment period.

CONCLUSION

The CIR Panel concludes that the available data are insufficient to support the safety of Disperse Yellow 3 for use in cosmetic products.

Acknowledgment: Bindu Nair, Scientific Analyst and Writer, prepared this report.

REFERENCES

Adams RM, Maibach HI, Clendenning WE, et al. (1985) A five-year study of cosmetic reactions. *J Am Acad Dermatol* 13:1062–9.

Corbett JF, Menkart J. (1973) Hair coloring. *Cutis* 12:190–7.

Cosmetic, Toiletry, and Fragrance Association (CTFA). (1993) Personal correspondence from G. N. McEwen. (dated: 12/6/93).*

Eiermann HJ, Larsen W, Maibach HI, et al. (1982) Prospective study of cosmetic reactions: 1977–1980. *J Am Acad Dermatol* 6:909–17.

Elder RL, ed. (1985) Final report on the safety assessment of *p*-phenylenediamine. *J Am Coll Toxicol* 4:203–66.

FDA. (1992) Frequency of use of cosmetic ingredients. *FDA database*. Washington, D.C.: FDA.

FDA. (1993) Frequency of use of cosmetic ingredients. *FDA database*. Washington, D.C.: FDA.

Foussereau J, Tanahaski Y, Grosshans E, Liman-Mestiri S, Khochnevis A. (1972) Allergic eczema from Disperse Yellow 3 in nylon stockings and socks. *Trans St. John's Hosp Dermatol Soc* 58:75–80.

Gray PS, Hunter R Jr., Patterson RM. (1979) Chromosomal aberrations induced by Dispersion Yellow 3 in *Rana clamitans* larvae during tail regeneration. *Cytobios* 25:175–82.

International Agency for Research on Cancer (IARC). (1990) Disperse Yellow 3. *IARC Monographs on the Evaluation of Carcinogenic Risk to Humans*. Vol. 48. Lyon: IARC, 149–59.

IARC. (1993) Occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. Vol. 57. Lyon: IARC, 43–118.

Ivett JL, Brown BM, Rodgers C, Anderson BE, Resnick MA, Zeiger E. (1989) Chromosomal aberrations and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. IV. results with 15 chemicals. *Environ Mol Mutagen* 14:165–87.

Kousa M, Soini M. (1980) Contact allergy to a stocking dye. *Contact Dermatitis* 6:472–6.

McGregor DB, Brown A, Cattanach P, et al. (1988) Responses of the L5178Y tk^{+}/tk^{-} mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. *Environ Mol Mutagen* 12:85–154.

National Toxicology Program (NTP). (1982) NTP technical report on the carcinogenesis bioassay of C.I. Disperse Yellow 3 (CAS NO. 2832-40-8) in F344/N rats and B6C3F_1/N mice (feed study). NIH Publication No. 82-1778. Bethesda: National Institutes of Health.

North American Contact Dermatitis Group. (1980) Patch testing in allergic contact dermatitis. Evanston: American Academy of Dermatology.

Oak Ridge National Laboratory. (1982) Chemical hazard information profile draft report C.I. Disperse Yellow 3 CAS No. 2832-40-8. (Submitted by EPA in response to an FOI request dated 3/22/93—24 pages).

Society of Dyers and Colourists. (1971) *Colour Index*, vol. 2 and 3. Bradford, England: The Society of Dyers and Colourists, Bradford, England. 484 (vol. 2); 3576 (vol. 3).

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