Search for benzidine and its metabolites in urine of workers weighing benzidine-derived dyes

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ABSTRACT Urinary benzidine and conjugates have been used to assess the extent of occupational exposure to benzidine-based azo dyes. The aim of the present study was to investigate the presence of benzidine and its conjugates in the urine of workers exposed to benzidine-derived dyes. Urine samples were collected from 29 workers in three textile dyehouses, two tanneries, and two dyestuff quality control laboratories in West Yorkshire and Lancashire. In 200 samples, obtained over a period of 15 months and analysed by gas chromatography/mass spectrometry, no free benzidine or monoacetylbenzidine was detected. Nevertheless, after strong acid hydrolysis of urine samples taken on two separate occasions from textile dye weighers where the standard of hygiene appeared below average, trace amounts of benzidine were detected suggesting the excretion of a benzidine derived dye or a metabolite thereof.

The benzidine-based azo dyes have attracted attention as suspect carcinogens,¹⁻³ and during an assessment of risks in woollen textile works in Yorkshire it became clear that a possible hazard area was the dyehouse weighing room. In these rooms powder dips, strong acids, and alkalis were used, often in a careless manner and often with poor facilities for personal hygiene. Even cooking and eating was permitted in certain premises.

Benzidine-derived dyes are not manufactured in Britain, and most dyes in use are imported from France, India, Poland, and Korea. Dyehouses using large quantities of these dyes were traced through the importers. In the Yorkshire/Lancashire areas tanneries and the cotton textile industry used most of these dyes; the wool textile producers using less. The coloured-paper industry also use quantities of benzidine-based dyes, but no such users were traced in these regions.

After a series of joint consultative meetings with dye manufacturers, dye users, and the union, a start was made on the preparation of a code of practice for dyehouse weighing rooms with emphasis on the careful handling of powder dyes to prevent inhalation, ingestion, and skin absorption.

It has been suggested that the carcinogenic risk arises not so much from residual free benzidine in these dyes but from benzidine generated during the

bleaching (stripping) of fabrics dyed by these substances, or the metabolic conversion of the dyes to free benzidine and monoacetylbenzidine in the body of workers exposed to a range of dyes, including the direct azo dyes. In this study we have looked for the presence of benzidine and its conjugates in the urine of workers handling (weighing) these dyes that might have arisen from free benzidine in the dyestuff or the metabolism in the body of the dyestuff.

Methods

The proposals for the study were discussed with workers, union representatives, and managements, which resulted in good co-operation. Over a period of 15 months urine samples were obtained from workers in textile factories (20), tanneries (5), and laboratories that tested imported dyes (4). Urine samples were collected early in the day before benzidine-derived dyes were weighed, later the same night, and first urine sample passed the following morning. The samples were frozen immediately and then taken to the laboratory for analysis.

ANALYTICAL METHODS

Two gas chromatographic mass spectrometric methods were devised for measuring benzidine and monoacetylbenzidine in urine. A VG Micromass 16F mass spectrometer linked to a VG 2035 data system and interfaced to a Pye Unicam 204 gas chromatograph via a glass jet separator was used in the

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Table 1 Exposure to benzidine-derived dyes and sampling details

	Maximum weight of dye handled a day (kg)	No of workers	No of urine samples
Laboratory technicians testing imported dyes	0.6	4	31
Textile dye weighers	30	20	114
Tannery dye weighers	50	5	64

Laboratory technicians use very small amounts, probably every day; tannery dye weighers use large amounts—up to 50 kg at a time—but maybe only once a week or once a month; and textile dye weighers use 3–5 kg at a time on most working days.

selected-ion monitoring mode. All urine samples were analysed for benzidine before and after hydrolysis (1M HCl) as the heptafluorobutyl derivative with methylene dianiline as an internal standard. The chromatograph was fitted with a 0.9 m × 2 mm glass column packed with 2% OV 17 on HP chromosorb W with helium at 25 ml/min as the carrier gas and an oven temperature of 240°C. The mass spectrometer operating conditions were: source temperature, 240°C; accelerating voltage, 4 kV; trap current, 200 μ A; electron energy, 70 eV; and scan speed, 1s/decade. A VG 8 channel digital multiple ion detector monitored characteristic ions for the heptafluorobutyl derivatives of methylene dianiline (m/z 590) and benzidine (m/z 576 and 379) with a lock mass from perfluorokerosene (m/z 581). The recovery of benzidine from urine was better than 90% and hydrolytic conversion of monoacetylbenzidine to benzidine was 95% efficient. The calibration for benzidine was linear over the range 5–540 nmol/l. The precision was better than 3% (coefficient of variation) at a concentration of 50 nmol/l and the detection limit was 5 nmol/l.

Table 2 Production of free benzidine in urine after acid hydrolysis

Subject	nmol benzidine/mmol creatinine*	
1	6.4	4.6
	6.0	4.2
2	6.6	
	9.5	8.4
	25.4	6.9
3	24.9	8.7
	2.8	2.9
	1.7	2.0
4	1.0	1.2
	8.9	6.8
	1.3	5.5
6	2.6	5.5
	8.6	13.8
	5.4	12.3
6-29	—	11.8
	None detected (n = 181)	

*The three figures represent three urine samples taken during same 24-hour period; two columns of data are from urine samples taken on separate visits to dye-house.

Electron impact (70 eV) mass spectrum of heptafluorobutyl derivative of benzidine. Ions m/z 379 and 576 are used in selected ion monitoring mode.

Samples yielding positive benzidine results after hydrolysis were reanalysed for monoacetylbenzidine as the isothiocyanate derivative⁴ on a 1.5 m × 2 mm mixed phase column (2% SP2100 and 2% SP2401) under isothermal conditions (270°C) with helium carrier flow at 30 ml/min. The molecular ion (m/z 268) and the base peak (m/z 226), which originates from loss of COCH₃ from the molecular ion, were used to detect the presence of monoacetylbenzidine with a detection limit of 45 nmol/l.

Results

Multiple urine samples were collected from 29 workers on several occasions (table 1). We were unable to detect free benzidine or monoacetylbenzidine in any of the 209 samples analysed. Urine samples from five workers from one woollen textile factory, however, were shown on two separate occasions to contain free benzidine after strong acid hydrolysis (table 2). The identity of the benzidine peak was confirmed by the presence of two characteristic mass spectral ions (m/z 576 and 379) (fig). On both occasions weighers of dyes were eating in the same room and seen to be sitting on open drums containing benzidine-based dyes.

Discussion

Mammals fed benzidine-derived azo dyes excrete a substantial proportion of the dyes as free benzidine or monoacetylbenzidine.^{2,3,5,6} These results are consistent with the known reduction of the dyes to benzidine by intestinal bacteria⁷ and with the finding that reduction of azo compounds can occur through the liver azo-reductase system.⁸ In this study we have been unable to find workers exposed to these dyes who excrete significant amounts of these two metabolites. We have, however, found free benzidine present after acid-hydrolysis of the urine of five workers. Possibly partial metabolic reduction or

breakdown of the dyes, or both, may give rise to larger molecular fragments than benzidine and its monoacetyl derivative, and strong acid hydrolysis results in the final breakdown of these materials or their conjugates to free benzidine. It is well established that small alterations in structure result in great changes in carcinogenic potential,⁹ and thus possibly this material present in the urine of the five workers represents no significant hazard. Since improvement in work-practices, these materials can no longer be detected in the urine. Hygienic practices, however, were clearly unsatisfactory in this particular workplace at the beginning of this investigation.

In this study we have shown that 24 out of 29 workers exposed to these dyes do not have detectable amount of benzidine or its conjugates in the urine. The other five workers excreted extremely small amounts of a benzidine-derived material that was not fully characterised. We suggest that these results give no direct cause for concern.

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