ENVIRONMENTAL LEVELS AND URINE CONTENT OF WORKERS EXPOSED TO AZO DYES

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ABSTRACT

Benzidine has been agreed to by both industry and government as being a proven human bladder carcinogen. Henceforth, the use of benzidine and the handling of it has either been curtailed or its exposure to workers greatly minimized. However, the azo dye products of benzidine have received little serious concern and have for at least 75 years been considered as being innocuous. Chemically and biologically, however, the azo bond is quite labile to reductive cleavage.

Because of recent suggestive evidence that these dyes may be broken down to their component amines in the body, NIOSH initiated field studies into the dye manufacturing and dye consuming industries where potential exposure to benzidine derived dyes were suspected. Both environmental and biological urine sampling was performed in order to evaluate actual exposure and the excretion of benzidine and its metabolites, hypothosizing that the dyes are metabolized to benzidine in vivo. The findings of this study are presented for each of the six field surveys performed and the results are discussed briefly.

Based on this and other evidence, NIOSH has recently recommended that benzidine derived azo dye be treated as carcinogens and their manufacture and use be discontinued.

INTRODUCTION

During the relatively brief history of the synthetic dye industry, benzidine has undoubtedly played a major role. Much of what we now know about dye chemistry was laid down between 1870 to 1910, a period which is sometimes called the classical period of dye chemistry. In 1884, P. Boettiger discovered Congo Red. This was the first direct cotton dye derived from benzidine (1). Since then more than 200 benzidine dyes have been listed in the Colour Index (2). In 1948, for instance, some four million pounds of benzidine and 31 million pounds of benzidine derived dyes were produced (3). This accounted for 21% of all dyes reported to be manufactured and almost all of the direct class dyes on the market in that year. While there is still a good demand for these dyes by industries, most manufacturing plants have stopped producing dyes made from benzidine, primarily because of environmental and health concerns. Today, there is only one domestic producer of these dyes; however, imports have risen appreciably. Benzidine derived dyes now used in the U.S. represent less than 1% of the at least 1200 different dyes made in the United States, and the additional 800 dyes imported (4,5). However, benzidine based Direct Black 38 has remained the single largest dye produced among all dyes.

During 1973 the proportional use of benzidine derived dyes, by industry, was estimated to be: 40% used to color paper, 25% to color textiles, 15% for leather, and 20% for diverse applications (6). They may be used

by crafts, artists, and the general public (7).

The object of the present study was to characterize the industrial environment in terms of worker exposure to benzidine derived dyes and to monitor their urinary excretion of benzidine and its metabolites.

A thorough literature review of the pertinent information on benzidine derived dyes has been recently published by NIOSH as a Special Hazard Review (8). In addition, the composite report for this study is to be published shortly (7).

STUDY DESIGN

Procedures had been developed for determining the concentrations of airborne dye exposure and for quantitating the urinary excretion of benzidine and its metabolites. It was desirable to locate industrial facilities where the probable exposure was relatively pure, e.g. where the primary chemical exposures during the work day were to benzidine dyes. With the cooperation of the American Textile Manufacturers Association and the Tanners Council of America, as well as other various sources of information, prospective facilities using benzidine dyes were queried and selected. In all, two textile dyeing and finishing facilities, a leather tanning facility and a specialty paper company were surveyed. The two benzidine dye manufacturing facilities operating at that time were also surveyed.

Employees in each facility were selected with the aid of the managements. Each employee who participated was monitored for personal airborne exposure and urinary excretion of benzidine.

ENVIRONMENTAL SAMPLING AND ANALYSIS

Personal airborne dyestuffs exposure was determined by sampling a known volume of air through a pre-weighed closed face 37-mm glass fiber filter in a three piece cassette. The sampled air was drawn through the filter by a calibrated personal sampling pump at approximately 1.8 liters per minute.

Bulk samples of the benzidine dyes used by the workers during the surveys were obtained for determination of residual-free benzidine present as both the base and salt.

Air filter samples were analyzed gravimetrically, which is a routine procedure designed to measure total gross particulates in the air. As a supplementary procedure for a quasi-specific method for identifying the proportional quantity of a benzidine dye on the same filter sample, NIOSH method P&CAM 234 was used (9). The principle of this method is that a sample filter is extracted with an appropriate solvent, and a spectrographic scan of the solution is performed in the 400-700 nm range. The absorbance maxima are compared to the absorbance maxima of standard

solutions prepared from the bulk samples of the azo dyes. This procedure was of use in the present study since only one to four different color dyes were used during most of the surveys over any one day.

Bulk samples of the dyes were analyzed for residual free benzidine and its salts using a liquid chromatographic procedure using a 280 nm UV detector. Studies have shown that this method yielded a recovery of approximately 100% for benzidine and its salts. The detection limit for benzidine is 1 ppm (w/w) from a one gram sample (7).

BIOLOGICAL SAMPLING AND ANALYSIS

Urine samples were collected in 180 mℓ polyethylene screw top bottles. The period of collection usually began during the beginning of the first shift that was monitored and continued (excluding the non-work period) into the middle of the next day. However, monitoring periods were extended to include up to a five day period, depending on the dye usage period. The time was recorded on each sample submitted, which was frozen immediately on dry ice and remained frozen until analyzed. Many samples were split so that they could be analyzed by both NIOSH at the Clinical and Biochemical Support Section, Division of Behavioral and Biomedical Sciences, and the Chemistry Division, National Center for Toxicological Research (NCTR).

Urines were analyzed by a screening colorimetric method adapted for use on human urines. The procedure was based on the pH5 extraction of aromatic amines from urine with chloroform, back extraction into HCL and reaction with 2,4,6-trinitrobenzene sulfonic acid (TNBS) to produce a yellow chromophore absorbing at 400 nm. The sensitivity of the method was 1 ppb based on a 100 ml urine sample. The presence of benzidine could be confirmed by thin layer chromatography if concentrations of benzidine exceeded 3 ppb. Complete details of the method have been published in Volume 5 of the NIOSH Manual of Analytical Methods (10).

Selected specimens were also analyzed by the electron-capture gas chromatograph method described earlier at this workshop in a presentation by Lowry and reported by Nony et. al. (11,12). This method was used to confirm the presence of specific metabolites and benzidine. The lower limit of detection for benzidine (Bzd) was 1.4 ppb and monoacetylbenzidine (AcBzd) 5.8 ppb. The method was also used to detect 3,3-dimethylbenzidine (DiMeBzd) and 3,3-dimethoxybenzidine (DiMxBzd) at a lower detection limit of 3 and 3.6 ppb, respectively. The identity of metabolites was confirmed by chemical synthesis of metabolites and derivatives followed by gas chromatography-mass spectrometry (GC-MS) analysis. Confirmation was also obtained to establish that these amines were in fact metabolites and not a result of chemical reduction of the intact dye by the analytical procedures.

RESULTS

Air filter samples were analyzed and reported as milligrams of total airborne particulate per cubic meter of air sampled. Detailed results of the environmental and spectrophotometric analysis on the samples are presented elsewhere (7).

Urine samples from 23 NIOSH office workers were submitted for analysis with the colormetric/thin layer chromatography procedure used to screen workers urine. Table I indicates that fewer than 35% excreted one or more ppb aromatic amine. No benzidine was detected. These results from this non-exposed group were useful for comparison purposes.

In the first dyestuff plant that was visited only spot urine samples were collected during a walk-through survey. Eight of thirty four dyestuff workers who were potentially exposed to the finished dye submitted urine samples. Using the screening method, less than 1 ppb aromatic amine was found in eight of ten workers. No benzidine was detected; however, 3 and 7 ppb monoacetylbenzidine was reported in two workers as shown in Table II. A return survey was not possible due to the discontinuance of benzidine dye manufacture at that facility.

The low level of aromatic amine excretion among these workers might be expected since cartridge filter respirators and local ventilation were commonly employed in this process.

Table II
Urinary Excretion in Dye Manufacturer I

Urine Specimen	Benzidine or Monoacetylbenzidine (MAB)	Aromatic Amines (ng/100 mL)	Thin-Layer Chromatography
1	3 ppb MAB	120	N.D.
2	N.D.	80	N.D.
3	N.D.	100	N.D.
4	N.D.	80	N.D.
5	N.D.	90	N.D.
6	N.D.	80	N.D.
7	7 ppb MAB	90	N.D.
8	N.D.	60	N.D.

N.D.--not detected

The second survey to the other dye manufacturing facility discovered the highest worker exposures of the entire study. A small company with minimal engineering control of the process, no formal respirator program, and no industrial hygiene or medical programs were implemented. Of about 55 production workers, about 34 were likely to be potentially exposed. While environmental monitoring data was collected in all dye finishing departments, employee cooperation was poor and only four provided intermittent urine samples. All urine samples contained benzidine and/or monoacetylbenzidine. Two workers also excreted 3,3'-dimethylbenzidine (o-tolidine) which was also used to make dyes. Environmental and urine monitoring results are tabulated for the four workers in Table III. Bulk samples of eleven dyes known or suspected to be benzidine derived were collected for residual benzidine analysis. All contained less than 20 ppm benzidine as the amine or salt.

The following results are from two dye finishing areas at two textile manufacturing facilities.

In the first facility surveyed, personal exposure concentrations were determined while the urine samples were analyzed by both the screening procedure and the EC-GC method. Benzidine and/or monoacetylbenzidine were found in three of seven workers who were monitored. Urinary concentration of non-specific aromatic amines were all above 1 ppb and generally considerably above the NIOSH comparison data. Table IV summarizes these results. Two bulk samples of the dyes used were found to contain 1 and 4 ppm residual total benzidine.

In the second textile facility visited, ten workers were monitored. None of the urine samples analyzed contain benzidine; however, one contained 4 ppb monoacetylbenzidine. The screening method indicated that 40% of the workers excreted one ppb or more aromatic amine in their urine. Environmental samples indicate that daily worker inhalation exposure in the dye room and otherwheres was less than 1.5 mg/m^3. Bulk samples of the dye contained up to 20 ppm (w/w) total benzidine. Table V summarizes the results of the monitoring data.

Employees at a leather facility were also monitored. Only Direct Brown 95 was used at the time of the survey. Three employees with the highest likely exposure were monitored as in the other facilities. None of the urine samples collected from the dye weigher or the two dye drum operators contained any detectable benzidine or monoacetylbenzidine. It is probable that appropriate work practices including wearing of respirators, eating in clean lunch facilities, using shower and wash facilities

after work, and changing out of work clothes after work probably contributed to these results. Sampling results are summarized in Table VI.

The last facility surveyed produced colored specialty paper. Black paper using 2500 pounds of Direct Black 38 was produced over a three day period. Urine samples were collected for up to five days from employees over all three work shifts each day. In all, 47 urine samples were submitted. Seven production workers participated from this facility. Environmental and urine monitoring data is summarized in Table VII. Workers I, III, and IV are dyestuff weighers, while the other four workers are operators of the pulp beaters. Like other dye using industries, these seven workers are probably the only ones on a regular basis to be directly exposed to dyes.

In addition to the analysis of benzidine and its metabolites in the urine of these workers, a recently discovered contaminant of Direct Black 38, diaminoazobenzene, was detected. Diaminoazobenzene (DAAB), also known as Basic Orange 2 or Chrysoidine, is an animal carcinogen. The dye used in the paper dyeing facility was not analyzed for DAAB. However, DAAB was found in the urine of four of the seven workers. Benzidine and/or monoacetylbenzidine was also found in four of seven of the workers, though concentrations were generally near the lower limits of detection. In addition, 57% of the urine samples submitted contained 1 ppb or greater non-specific aromatic amines. This is a higher percentage than was found in the NIOSH comparison group.

It should be noted that the above workers who weighed dyes did so while wearing a half face NIOSH approved respirator. Exhaust ventilation near the dye weighing scales was also utilized to lower exposures. Airborne concentrations were generally below 5 mg/m^3, while actual exposure would have been less when using a respirator.

CONCLUSIONS

In total, urine samples were collected over varying lengths of time from 38 industrial employees who were regarded as potentially exposed to benzidine derived dyes. Of that number, benzidine or monoacetylbenzidine in quantities ranging from one part per billion to 112 ppb benzidine and 590 ppb MAB were found in 12 of the 38 workers monitored. Environmental exposures and work practices were recorded in an attempt to associate these factors with biological excretion.

Evidently, benzidine derived dyes can be used in the work place without detecting benzidine or its metabolites in the urine. By comparing the results from the six facilities surveyed it appears that total airborne particulate concentrations above 3-5 mg/m^3 frequently resulted in detecting benzidine, its metabolites, or elevated, non-specific aromatic amines in the workers urine. Worker exposure to airborne particulates less than 3 mg/m^3 are less often associated with finding aromatic amines in the urine. However, full shift airborne exposures as low as 1.1 mg/m^3 resulted in considerable benzidine in some workers urine, thus suggesting the difficulty in providing sufficient controls.

Piotrowski (13) has provided evidence that urinary benzidine concentrations of 100 ppb or greater are associated with an elevated risk of bladder cancer in man. In addition, the National Cancer Institutes recent testing of three benzidine derived dyes Direct Black 38, Direct Brown 95 and Direct Blue 6 indicate that the benzidine derived dyes may be more carcinogenic in the rat than benzidine alone (14). In view of this and other recently published information on these dyes, it is difficult to see how they can be used in a sufficiently controlled and safe manner. Therefore, NIOSH has recommended that benzidine derived dyes be treated as carcinogens and that steps be taken to substitute or minimize employee exposure as much as possible.

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Table I. NIOSH control urine results.

Individual	Aromatic Amines* (ng/100ml)	Approximate ppb	TLC** Confirmation
1++	205	2	ND
2	ND	< 1	ND
3	ND	< 1	ND
4	ND	< 1	ND
5	100	1	ND
6++	ND	< 1	ND
7	ND	< 1	ND
8	120	1.2	ND
9+	300	3.0	ND
10	ND	< 1	ND
11	ND	< 1	ND
12	ND	< 1	ND
13	ND	< 1	ND
14	ND	< 1	ND
15++	ND	< 1	ND
16	ND	< 1	ND
17	ND	< 1	ND
18	145	1.4	ND
19	100	1.0	ND
20	100	1.0	ND
21	200	2.0	ND
22	ND	< 1	ND
23	ND	< 1	ND
Standard 1 (Bzd)	100	1.0	Positive
Standard 2 (Bzd)	250	2.5	Positive
Standard 3 (Bzd)	350	3.5	Positive

*Expressed as benzidine.
**Thin-layer chromotography
+Allergy medication taken
++Pipe smokers

Note: Lower limit of detection is 100 ng/100 mL of urine.

Table III. Environmental and biological sampling data from Dye Manufacturer--Facility B.

Job Description	Daily Personal Exposure	Urinary Excretion*	Notes
Pulverizer 1	Day 1) 12 mg/m^3 total 2) 5.9 mg/m^3 total 2.5 mg/m^3 respirable	52 ppb Bzd†; 248 MAB 18 ppb Bzd	Wore cartridge respirator. Occasional exposure to very high levels during adjustments
Spray dry	1) 17.4 mg/m^3 - area near chute	112 ppb Bzd; 590 ppb MAB: 50 ppb DiMeBzd	Wore no respirator - most of day spent outside building. The presence of DiMeBzd would indicate previous exposure as no DiMeBzd dyes were being used on day of sampling.
Pulverizer 2	1) 6.2 mg/m^3 total 2) 14.1 mg/m^3 total	10 ppb aromatic amines expressed as Bzd; Bzd confirmed on TLC 5 ppb aromatic amine as benzidine	Wore cotton gauze respirator; became very dirty from dyestuffs worked mostly with a non Benzidine dye.
Tray Oven	1) 7.0 mg/m^3 total 2) 6.5 mg/m^3 total	11 ppb Bzd; 22 ppb MAB; 15 ppb, DiMeBzd	Wore cartridge dust respirator; emptied dried oven trays into drums

+ Abbreviations:
Bzd - benzidine
MAB - monoacetylbenzidine
DiMeBzd - 3,3' - dimethylbenzidine

* Limit of Detection (ppb)
Bzd - 1.4
MAB - 5.8
DiMeBzd - 3.0

Table IV. Environmental and urinary excretion in Textile Facility C

Workers Personal Exposure	Urinary Excretion*			Notes
	Aromatic Amines	Benzidine	Monoacetyl-benzidine	
1) 1.39 mg/m^3	3.2 ppb 4.4 ppb	ND	ND	Dye weigher wore no respirator. General ventilation only.
2) 1.06 mg/m^3	8 ppb	benzidine confirmed by TLC		Dye weigher wore no respirator. General ventilation only. Boiled up dye by hand.
	5.8 ppb	39 ppb	5 ppb	
3) 1.68 mg/m^3	5 ppb	1 ppb	7 ppb	Dye weigher wore no respirator. Beginning of shift-dustiest
4) 2.06 mg/m^3	3 ppb	ND	ND	Dye weigher wore no respirator. Beginning of shift dustiest.
5) 1.07 mg/m^3	4.5 ppb	ND	ND	Pad dye operator carried dye solution to dye baths and diluted to desired conc. Spent 80% of time in non-exposure area loading gray goods into Pad Dyeing Machine. Wore no respirator. No contact with dry dye.
6) 1.12 mg/m^3	9 ppb	benzidine confirmed by TLC		Pad Dyeing Operator
	13 ppb	16 ppb	38 ppb	
7) 1.98 mg/m^3	3.4 ppb 3.0 ppb 4.0 ppb 3.2 ppb	 ND ND	 ND ND	Jigg Dyer, wore no no respirator. Spent much time near steamy jigg baths making adjustments on cloth rolls. No contact with dry dyes.

Table V. Results of urine and environmental monitoring at Facility D- a textile dyer.

Personal Worker Exposure*	Urinary Concentrations			Notes
	Aromatic Amines	Benzidine	Monoacetyl-benzidine	
1) 1.54 mg/m^3 1.31 mg/m^3 1.45 mg/m^3 TWA	4 ppb 4.8 ppb 3.6 ppb	ND ND	4 ppb ND	Dye Weigher Weighed dyes in Drug Room before dissolving in boil-up tubs. Worker sometimes wore a half-face pad type respirator and gloves when weighing dyes. General ventilation from roof exhaust fans only.
2) 1.15 mg/m^3 1.11 mg/m^3 1.13 TWA	ND ND 1.3 ppb	ND	ND	Same as above
Area sample over scales 0.55 mg/m^3				
3) 5.31 mg/m^3 (void)	ND ND			Dye Tub Operator Worker loads and unloads cloth from rolls to and from dye tubs. Tubs were ventilated by top hood exhaust and had front hood moveable doors. No respirators worn. Rubber gloves worn sometimes. Worker is splashed by dye liquor during work.
4) 0.90 mg/m^3	ND			Same as above
5) 1.58 mg/m^3	3.2 ppb			Same as above
6) 0.67 mg/m^3	ND			Same as above
7) 0.63 mg/m^3	3.2 ppb			Same as above
8) 0.20 mg/m^3	ND ND ND			Same as above
9) 0.60 mg/m^3	ND			Same as above
10) 0.48 mg/m^3	ND			Roll-up Machine Operator worker operates steam-press roll-up machine. Considerable heat and resultant steam evolved. No respirator worn.

*Environmental concentrations expressed as total airborne particulates per cubic meter of samples air.

Table VI. Dye Exposure among three workers at a Leather Tannery--Facility E.

Personal Worker Exposure	Urinary Excretion	Notes
1) 12.05 mg/m^3 2) 12.95 mg/m^3 3) 14.72 mg/m^3 4) 1.27 mg/m^3 10.65 mg/m^3 TWA*	Day 1 ND+ ND Day 2 ND ND	Dyestuff Weigher Only benzidine-derived C.I. Directo Black 38 and C.I. Direct Brown 95 were used. Dye weigher spent 80% of time weighing these dyes into paper bags. Half-face cartridge respriator was worn during weighing.
5) 1.42 mg/m^3 6) 0.44 mg/m^3 7) 0.00 0.69 mg/m^3 TWA	Day 1 ND ND Day 2 ND ND	Dye Drum Operator I Operator picks up bagged dyes from weigher and empties bag into solvating tub. Potential exposure would only occur during emptying. Both operators wore half-face cartridge respirators during dye handling. Other responsibilities include loading and unloading dyed hides from dye bin.
8) 1.12 mg/m^3 9) 1.65 mg/m^3 10) 16.79 mg/m^3 5.79 mg/m^3 TWA	Day 1 ND ND Day 2 ND ND	Same as above

*Time Weighted Average
+Benzidine not detectable

Table VII. Results of environmental and urine monitoring at Paper Facility F

	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	Env. Conc.* (mg/m^3)	Urine (ppb)	Env. Conc. (mg/m^3)	Urine (ppb)	Env. Conc. (mg/m^3)	Urine (ppb)	Env. Conc. (mg/m^3)	Urine (ppb)	Env. Conc. (mg/m^3)	Urine (ppb)	Env. Conc. (mg/m^3)	Urine (ppb)
Worker 1	-	N.D+ (N.D)++	-	N.D (N.D)	3.3	N.D (1.4)	3.4	3 MAB (1)	Void	3 MAB 1 Bzd 32 DAAB (4.9)	-	8 MAB 2 DAAB
Worker II	-	N.D	-	N.D (N.D)	2.3	N.D -	5.1	N.D -		1 Bzd 5 DAAB (2.9)		N.D
Worker III	-	N.D (1.0)	-	N.D (2.0)	1.6	N.D. 2.2	2.5	N.D -	-	N.D (2.6)		
Worker IV	-		-	N.D (N.D)	3.7	N.D (1.3)	Void	N.D		(N.D) (N.D)		
Worker V				N.D (1.0)	2.9	N.D (N.D)	2.3	N.D -	-	2 DAAB (N.D)		
Worker VI			-	N.D (1.3)	2.6	N.D (1.3)	Void	2 MAB -	-	2 MAB		2 MAB
Worker VII								3MAB 3DAAB		2MAB		

FOOTNOTES:

* environmental concentrations expressed as milligrams total airborne particulates per cubic meter air.

\+ concentration of specific aromatic amines in ppb with the following detection limits: benzidine 1.0ppb; MAB, 1.8ppb; DAAB, 0.8ppb; diacetylbenzidine, 0.2ppb

++ concentration of non-specific colorimetric procedure with the limit of detection at 1ppb.

NOTE: Area within heavy black line signifies period of Direct Black 38 useage.

Dr. Weisburger (NCI): We have time for one or two questions. Yes?

Dr. Landrigan (CDC): Did you analyze bulk samples of the dye or samples of the airborne particulates that you captured on your filters to see whether or not there was benzidine in that material, or are you convinced that the benzidine which was detectable in the urine arose entirely by metabolism within the bodies of workers?

Mr. Boeniger (NIOSH): This was an important step in the research protocol. In every case where benzidine-derived dyes were used in the work place, the dyes were collected and analyzed for residual free benzidine content.

We have summarized considerable data on domestically produced benzidine dyes and imported derived dyes for their content of residual benzidine.

At the levels of residual free benzidine in these dyes, we tried to calculate from historical information what the likely excretion from simply the residual quantity of benzidine was, not accounting for possible metabolism of the dyes themselves, and in all cases, even with leniency, the levels of excretion would have been below the minimal level of detection of our procedures.

So while we have not been able to prove the metabolism of the Direct Black 38 or any of the other benzidine-derived dyes, it is highly suggestive that this work is in agreement with the animal experiments which have demonstrated metabolism of the benzidine dyes.

Dr. Lanrigan (CDC): Have OSHA or the Department of Commerce picked up on the NIOSH recommendation to limit or ban the use of this material, and are they working on regulations either to restrict industrial use or to forbid the importation?

Mr. Boeniger (NIOSH): At this time, my understanding is that they have not taken the position of eliminating them or banning the benzidine-derived dyes, but simply to minimize the exposure to lowest feasible levels.

Dr. Saffotti (NCI): I wanted to ask you something about the presence of several aromatic amines concurrently. You have indicated, and I am sorry I missed the paper given by Dr. Lowry on the previous session, that you find benzidine and 4-aminobiphenyl and some other compounds as a breakdown product of the dyes.

We have been doing some work in in vitro systems on combined effect of various carcinogens, and one of the series we have recently studied is a series of aromatic amines, for which we have gotten a number of data in the Ames test, which again I would like to eventually compare with those that were reported by Dr. Lowry.

In studying the combinations of various aromatic amines together, we have found a number of them inhibiting each other in the Ames test and on occasion some synergism. One of the combinations that seems to be repeatedly postive as synergistic is benzidine and 4-aminobiphenyl.

In comparing the mutagenic activity of the Direct Black 38 with that of benzidine or 4-aminobiphenyl alone, one certainly finds a much higher level of activity of Direct Black 38 than the individual compounds, although I would like to have eventually some discussion about the solvent systems used and other things like that.

In relation to the monitoring in the human, do you have evidence of 4-aminobiphenyl or other compounds being detectable in the urine and possibly their concurrent presence with benzidine?

Mr. Boeniger (NIOSH): Initially, our methodologies were rather simple, but as we progressed into the last survey, and only in the last survey were we actually looking for other metabolites or other aromatic amines other than benezidine or its metabolites -- for instance, 4-amino-biphenyl -- and in the last facility we did not find 4-aminobiphenyl in any of the workers' urines there. So that was the only indication where they were actually looked for.

We did find it using Direct Black 38 in animal experiments and just do not know how to correlate that right now.

Dr. Jenkins (EPA): What other facilities have you now monitored over several days trying to establish some of the kinetics of the excretion patterns of benzidine besides that one paper facility?

Mr. Boeniger (NIOSH): Well, that was the last one in our study. We had at that time run out of both time and funds anticipated for the completion of this study.

There were some surveys previous to that where we looked at workers during the day of exposure and the following day of exposure, so there was a 2-day follow-up period there.

Dr. Jenkins (EPA): But no other long exposures like that paper?

Mr. Boeniger (NIOSH): No, I am afraid not.

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