

## VIII. REFERENCES

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## APPENDIX I - ANALYSIS

### (A) METHOD FOR MONITORING DIAZONIUM SALTS AND AZO DYES IN AIR

A screening method for analysis of benzidine-based dyes found in the workplace has been developed by NIOSH [71] and is presented below. A negative survey using this methodology is considered adequate to establish that workers are not significantly exposed to the benzidine-based dyes.

Analyte: Diazonium Salts  
and Azo Dyes

Method No.: P&CAM 234

Matrix: Air

Range: 0.01 to 0.4 mg/cu m in a  
500-liter sample of air

Procedure: Filter collection;  
UV-VIS spectrophotometry

Precision (CV): 0.12  
(analytical)

Date Issued: 6/30/76

Classification: D(Operational)

#### Principle of the Method

Duplicate samples of airborne particulate material are collected on cellulose acetate membrane filters. One filter is extracted with dilute hydrochloric acid and analyzed for diazonium salts spectrophotometrically at 375 nm. The second filter is extracted with an appropriate solvent and a spectrophotometric scan of the solution is made in the 400- to 700-nm range. The absorbance maxima are compared with the absorbance maxima of standard solutions prepared from bulk samples of the azo dyes.

#### Range and Sensitivity

- (a) Diazonium salts and azo dyes follow a Beer's law relationship in the range of 5-200  $\mu\text{g}$  in 40 ml of solvent, the volume used to extract the filters. This corresponds to 0.01-0.4 mg/cu m in a 500-liter air sample.
- (b) Dilution of the sample solution with the appropriate solvent may be used to extend the upper limit of the range.
- (c) The lower limit of the range can be extended by decreasing the volume of the extracting solvent or by using an absorption cell of longer length.



- (d) The sensitivity is determined by the absorptivities of the individual salts and dyes.

#### Interferences

Any compound that absorbs radiation at the wavelengths corresponding to the absorbance maxima of the diazonium salts and azo dyes is a positive interference.

#### Precision and Accuracy

- (a) An average coefficient of variation of 0.12 has been established for the analytical procedure over the concentration range of the method.
- (b) The recovery of salts and dyes from filters with the appropriate solvent yielded an efficiency of  $95 \pm 5\%$  in the range of 5-200  $\mu\text{g}/40$  ml of solvent.
- (c) The accuracy of the overall sampling and analytical method has not been determined.

#### Advantages and Disadvantages

- (a) The sampling procedure is rapid, simple, and involves no liquids.
- (b) The procedure can be used to distinguish between diazonium salts and azo dyes but not between two salts or two dyes absorbing at the same wavelength.
- (c) Since diazonium salts exhibit maximum absorption at approximately 375 nm, the method cannot be used for qualitative identification of individual salts. Thus the total diazonium salt concentration must be reported if more than one salt is being processed in the testing area on the day of sampling. Since each salt has a characteristic absorptivity, the concentration of total diazonium salts must be given as a range. The salt having the highest absorptivity determines the upper limit of the range and the salt having the lowest absorptivity determines the lower limit.
- (d) Diazonium salts deteriorate in the presence of light and moisture. Care must be taken to store them in a dry environment and to minimize exposure to light.
- (e) Bulk samples of all of the diazonium salts processed in the testing area on the day of sampling must be collected and a calibration curve must be established for each.

- (f) The solubilities of the various classes of azo dyes differ widely. Therefore, an appropriate solvent for each azo dye must be determined. Azo dyes that contain one or more sulfonic or carboxylic acid groups are usually soluble in dilute acid. Azo dyes with sulfonated naphthol groups are usually soluble in water or dilute base. Azo dyes with no solubilizing groups are usually insoluble in water but are soluble in polar or nonpolar organic solvents.
- (g) Absorption curves must be established for all azo dyes processed in the plant on the day of sampling. Azo dyes exhibit maxima in the 400- to 700-nm range, the exact wavelength being dependent on the structure of the dye molecule.
- (h) If more than one azo dye is being processed on the day of sampling and those dyes absorb at a common wavelength, the total azo dye concentration must be reported. The concentration of total azo dye must be given as a range with the dye exhibiting the highest absorptivity determining the upper limit and the dye exhibiting the lowest absorptivity determining the lower limit at a specific wavelength.

#### Apparatus

- (a) Air Sampling Equipment
  - (1) Cellulose ester membrane filters, 0.8- $\mu$ m pore size, 37-mm diameter, Millipore Type AA, or equivalent.
  - (2) Filter holder for 37-mm filters, Millipore MAWP 037 A0, or equivalent.
  - (3) Personal sampling pump, calibrated with a representative filter unit in the line. A wet or dry test meter or a glass rotameter capable of measuring a flowrate of 2 liters/min to within 5% may be used in the calibration.
- (b) Beckman Model 25 Scanning Spectrophotometer or equivalent.
- (c) Matched fused silica cells, 1, 5, and 10 cm.
- (d) Balance capable of weighing accurately to 1 mg.
- (e) Pipets, various sizes.
- (f) Beakers, 100 ml.

## Reagents

- (a) Hydrochloric acid, 0.1 N (pH = 0.9).
- (b) Bulk samples of all diazonium salts and azo dyes processed in the testing area on the day of sampling.
- (c) Appropriate solvents for dissolving the azo dyes.
  - (1) Toluene, ACS reagent grade.
  - (2) Distilled water.
  - (3) pH 5 buffer solution. Prepare by adding potassium hydrogen phthalate to distilled water and adjusting the pH to  $5.0 \pm 0.2$  with a calibrated pH meter.
  - (4) pH 9 buffer solution. Prepare as above with ammonium acetate, adjusting the pH to  $9.0 \pm 0.2$ .

## Procedure

- (a) Cleaning of equipment. Wash all glassware in hot detergent solution and rinse well with hot tap water, then rinse several times with double distilled water.
- (b) Collection and Shipping of Samples
  - (1) Place a clean filter and backup pads into the cassette filter holder.
  - (2) Connect the cassette to the vacuum pump. No tubing should be placed in front of the filter.
  - (3) Turn on the pump to begin sample collection. Measure the flowrate and time, or volume, as accurately as possible. Sample 500 liters or more at 1.5-2 liters/min.
  - (4) Take duplicate samples at each sampling site. One is for the diazonium salt analysis; the other for the azo dye analysis. Protect the filters from light to prevent photodecomposition of the diazonium salts.
  - (5) Ship the filters in a suitable container designed by NIOSH to minimize contamination and to prevent damage in transit. Include two or more blank filters, which are handled in the same manner as the sample filters except that no air is sampled through them.

(c) Analysis of Samples

(1) Diazonium Salts

Extract one of the duplicate sample filters from each site with 40 ml of 0.1 N HCl in a 100-ml beaker. Agitate the mixture in an ultrasonic bath for 5 minutes. Allow the filter and any suspended solids to settle. Extract a blank filter in the same manner. Perform the extractions with minimum exposure to light. Transfer a portion of each solution to a 1-, 5-, or 10-cm fused silica cell, depending on the anticipated diazonium salt concentration. Determine the absorbance of the blank and the sample of 375 nm. Use 0.1 N HCl as the reference solution.

(2) Azo Dyes

Select an appropriate solvent by experimentation with the bulk samples. (See Advantages and Disadvantages, section (f).) Extract one of the duplicate sample filters and a blank filter with the solvent using the extraction procedure outlined in section (1) on Diazonium Salts. Transfer a portion of each solution to a 1-, 5-, or 10-cm fused silica cell, depending on the anticipated concentration. Using the solvent as a reference, scan the 400- to 700-nm range to locate the absorbance maxima. Measure the absorbance at each wavelength where the bulk azo dyes absorb. (See Calibration and Standards (b) Azo Dyes.)

Calibration and Standards

(a) Diazonium Salts

- (1) Prepare a set of five standards for each diazonium salt that may be present in the sample. Each set should consist of standards containing 5, 25, 50, 100 and 200  $\mu\text{g}$  of one diazonium salt in 40 ml of 0.1 N HCl.
- (2) Transfer a portion of each solution to a 1-, 5-, or 10-cm fused silica cell.
- (3) Using the 0.1 N HCl solution as the reference solution, determine the absorbance of each standard at 375 nm.
- (4) Construct a standard curve for each diazonium salt by plotting the absorbance against the amount (in micrograms) of the individual diazonium salt in the corresponding standard.

(b) Azo Dyes

- (1) Prepare a set of standards for each azo dye as in Section (a) (1) using the appropriate solvent.
- (2) Transfer each solution to a 1-, 5-, or 10-cm fused silica cell.
- (3) Scan the spectrum of one standard solution for each different dye to determine the wavelength of maximum absorption. Use the solvent as the reference solution.
- (4) Determine the absorbance of each standard at the wavelength of its maximum.
- (5) Construct a standard curve for each azo dye by plotting the absorbance against the amount (in micrograms) of the individual azo dye in the corresponding standard.

Calculations

(a) Diazonium Salts

- (1) If only one diazonium salt is present in the sample, read from the appropriate calibration curve the amount (in micrograms) corresponding to the absorbance of the sample.
- (2) If more than one diazonium salt is present, the results must be given as a range. (See Advantages and Disadvantages (f).) From the calibration curve for each diazonium salt, read the amount (in micrograms) corresponding to the absorbance of the sample. The upper limit of the range is taken from the curve yielding the largest value. The lower limit of the range is taken from the curve yielding the lowest value.

(b) Azo Dyes

- (1) If only one azo dye is present in the sample, read from the appropriate calibration curve the amount (in micrograms) corresponding to the absorbance of the sample at the wavelength of maximum absorption.
- (2) If more than one azo dye is present, the results must be given as a range. (See Advantages and Disadvantages (h).) From the calibration curve for each azo dye absorbing at the same wavelength as the sample, read the amount (in micrograms) of azo dye corresponding to the absorbance of the sample. The upper limit of the range is taken from the curve

yielding the largest value. The lower limit of the range is taken from the curve yielding the lowest value.

- (c) Correct the calculated amount or range of amounts of the appropriate diazonium salt(s) or azo dye(s) for any corresponding value found by the analysis of blank filters.
- (d) The concentration or range of concentrations of diazonium salts and azo dyes may be expressed in mg/cu m:

$$\text{mg/cu m} = \frac{\text{Amount ((ug)g)}}{\text{Vs (in liters)}}$$

where: Vs = volume (liters) of air sampled.

## APPENDIX I - ANALYSIS (CONTINUED)

### (B) METHOD FOR MONITORING FOR BENZIDINE IN URINE

NIOSH has reviewed the various methods of biological monitoring for benzidine and related chemicals. There are two acceptable methods: the recommended method that follows [79] and the fluorescamine method given in the o-tolidine criteria document [73]. The recommended method presented here is less expensive and more sensitive than the fluorescamine method; however, it requires a longer working time. Neither method is specific for benzidine.

Any other method of at least equal sensitivity and precision may be substituted for the recommended method [37,59,78].

#### Aromatic Amines in Urine BENZIDINE IN URINE (SCREENING TEST)

<u>Analyte:</u>	Aromatic Amines (as Benzidine)	<u>Range:</u>	100-20,000 ng/ 100 ml urine
<u>Matrix:</u>	Urine	<u>Precision:</u>	Not determined
<u>Procedure:</u>	CHCl <sub>3</sub> extraction HCl reextraction Spectrophotometry (Benzidine confirmation by thin-layer chromatography)	<u>Classification:</u>	D (operational)
		<u>Date Issued:</u>	8/3/79

#### Principle of the Method

Aromatic amines, including benzidine, are extracted from urine with chloroform after pH adjustment. The chloroform extract is back extracted into 0.1 N HCl and derivatized with 2,4,6-trinitrobenzene sulfonic acid (TNBS). The TNBS derivatives are extracted with chloroform to remove interfering chromophores and the final chloroform extract quantitated spectrophotometrically at 400 nm while using benzidine as a standard. The resulting chloroform extract is concentrated to droplet volume for benzidine identification by thin-layer chromatography (TLC). Attention is called to the poor stability of the chloroform solution of the TNBS derivative of benzidine. Quantitation of this derivative by colorimetry should be performed within 30-45 minutes after preparation of the final chloroform solution.

### Range and Sensitivity

(a) The detection limit of this spectrophotometric procedure is 100 ng of benzidine (aromatic amine) per 100 ml of urine. The detection limit for benzidine by TLC is 300 ng/100 ml urine.

(b) The range is from the detection limit up to 20,000 ng/100 ml of urine.

(c) The UV and/or visible detector (maximum absorbance, 400 nm) has a linear response up to 0.02 mg of benzidine per 100 ml of urine.

### Interferences

(a) Any other aromatic amine having an absorbance maximum at 400 nm and an R<sub>f</sub> value identical to that of benzidine when chromatographed by TLC would interfere.

(b) Aromatic amines in substances that are normally present, ingested, or produced by metabolic processes in the worker will produce a chromophore (false positive).

(c) Extraction at pH 5 with chloroform and subsequent back extraction into HCl reduce interference from other compounds.

### Precision and Accuracy

(a) Recovery studies conducted on benzidine indicate 70% at the 500 ng/100 ml level and a marked decrease (about 20%) at the 100 ng/100 ml level.

(b) The precision has not been evaluated at this time.

### Advantages and Disadvantages

(a) The principal advantage is that the method is specific for aromatic amines in the nanogram range.

(b) Another advantage is that benzidine at 300 ng/100 ml can be confirmed by TLC in conjunction with the method. It should be noted that the TLC confirmation data does not prove that the chromophore is benzidine. More rigorous methods must be used for absolute confirmation.

(c) The disadvantages of the method are the complexities of the procedure (emulsions and losses in extractions), nonspecificity for benzidine, and the increased time for confirmation by TLC.



(d) A rigorous specific method for benzidine in urine has recently been published [37,59]. It uses fluoroanhydride derivatization and electron capture gas chromatography, but requires considerable more time and equipment to perform.

### Apparatus

(a) Spectrophotometer capable of measuring absorbance at 400 nm and accommodating semimicrocuvettes (1-ml capacity).

(b) Centrifuge with speed range to 4000 rpm.

(c) Rotator for mixing test tubes (25x200 mm).

(d) pH meter.

(e) TLC plates precoated with silica gel and without fluorescence indicator (0.5-mm thickness, E. Merck, Darmstadt, Germany).

(f) Chromatographic tank for thin-layer chromatography.

(g) UV source for reading TLC plates.

(h) Graduated cylinders (100 ml).

(i) Glass bottles with Teflon-lined caps (180-ml capacity, 6-ounce size).

(j) Volumetric glass pipets (2 and 5 ml).

(k) Separatory funnels (125 ml).

(l) Volumetric flasks (25 and 100 ml) and a 10-ml amber flask.

(m) Glass culture tubes with Teflon-lined caps (16x125 mm and 25x200 mm).

(n) Microliter pipets (0.01, 0.1, and 0.7 ml).

(o) Nitrogen source for concentrating organic samples.

(p) Disposable Pasteur glass pipets and bulbs.

(q) Disposable plastic gloves.

(r) Desiccator.

(s) Polyethylene bottles, 250 ml for urine collection.

Reagents (All reagents must be ACS reagent grade except where otherwise noted.)

(a) Benzidine, 99% (available from RFR Corporation, 1 Main Street, Hope, Rhode Island 02831) (CAUTION: CARCINOGEN).

(b) Chloroform.

(c) 2,4,6-Trinitrobenzene sulfonic acid (TNBS) (Eastman Organic Chemicals, reagent grade). 100 mg TNBS per ml of water. Stable for up to 7 days in the dark.

(d) Methyl alcohol.

(e) Sodium hydroxide, 1 N.

(f) Sodium chloride.

(g) Sodium acetate buffer pH 5.5, 2 M (refrigerate).

(h) Hydrochloric acid, 1 N and 0.1 N.

(i) Acetone.

(j) Formic acid.

### Procedure

#### (a) Cleaning of Glassware

(1) All glassware used for the laboratory analysis should be treated with chromic acid, rinsed with tap water and washed in detergent. The glassware is rinsed thoroughly in distilled water and air dried.

(2) Samples of "spot" urine (150 ml) are collected following 6 hours of suspected exposure to benzidine-based azo dyes.

(3) Samples should be collected in polyethylene bottles and, if not analyzed on the same day, should be frozen until analysis can be done.

#### (b) Analysis of Samples

(1) One hundred milliliters of well mixed urine is adjusted to pH 5.0 to 6.0 (1 N HCl or 1 N NaOH) in a glass bottle (180-ml capacity). A control urine sample (100 ml) and a control urine sample (100 ml) spiked with benzidine (300-1000 ng) should be analyzed concurrently with the unknown samples.

(2) Add 0.2 g NaCl crystals to the pH adjusted urine.

(3) The urine is extracted with 10 ml of chloroform for 2 minutes. If an emulsion is formed, then centrifuge to separate the two phases. The chloroform fraction (organic phase) is collected and saved.

(4) The urine is extracted twice more with chloroform (10 ml), and all three of the chloroform fractions are combined.

(5) Reextract the combined chloroform mixture with 2 ml of 0.1 N HCl for 30 minutes on a rotator.

(6) Transfer the aqueous phase (about 2 ml) into a culture tube (16x125 mm) using a Pasteur pipet.

(7) Add 2 ml of pH 5.5 buffer and 0.7 ml of TNBS reagent, mix well, and let stand for 15 minutes at room temperature. A reagent blank is prepared by adding 2 ml of 0.1 N HCl to 2 ml of pH 5.5 sodium acetate buffer and 0.7 ml TNBS reagent and is treated as a sample.

(8) Add 2 ml of  $\text{CHCl}_3$  and shake for 1 minute.

(9) Measure the absorbance of the organic phase at 400 nm on a spectrophotometer.

(10) Retain the organic phase for the benzidine-TLC confirmation.

#### (c) TLC Confirmation of Benzidine

(1) The TNB-derivative (chloroform extract) is concentrated by evaporating with nitrogen to about 0.2-ml aliquot.

(2) Ten microliters of the aliquot is spotted on a silica-gel TLC plate that was activated at 110 C for 30 minutes.

(3) The plate is then developed in chloroform-formic acid 90:10, volume to volume (prepared daily).

(4) The  $R_f$  of the unknown amine derivative is compared with that of a benzidine spiked derivative, which should always be run as a standard. Benzidine produces a spot on the TLC plate having an  $R_f = 0.41$ , visualized by both visible and UV light. The spot is yellow in visible light and appears as a dark spot under UV light.

#### Calibration and Standardization

(a) CAUTION: Benzidine is a known human carcinogen and appropriate precautions should be utilized to minimize exposure. All wastes including acetone rinsed dirty glassware should be collected and disposed by approved methods.

(b) Prepare a working standard solution containing 10  $\mu\text{g}$  of benzidine per ml of methyl alcohol. A series of benzidine spiked urine samples are prepared from a urine pool sample that was previously shown to have less than 100 ng benzidine per 100 ml of urine. The spiked urine samples serve as standards and are analyzed by the colorimetric method. The calibration curve is established by plotting benzidine concentration (ng per 100 ml of urine) vs the absorbance at 400 nm.

#### Calculations

(a) The concentration of the analyte in the urine sample is compared with a standard curve prepared with benzidine spiked urine samples as described in Calibrations and Standardization. All samples are read against a reagent blank as described in Procedure.

(b) No corrections for extraction efficiency are needed since standards are prepared in urine and both standards and samples are treated the same way.

(c) If the calculated concentration exceeds 300 ng/100 ml, the chloroform extract should be analyzed by TLC to tentatively confirm the presence of benzidine.

(d) In this laboratory, normal rangds of urine specimens from NIOSH employees not exposed to benzidine or aromatic amines are reported below:

<u>Number of Urine Specimens</u>	<u>Aromatic Amine Conc. (ng/100 ml)</u>
10	less than 100
2	100 - 120
1	200
1	300

Total Number: 14.

Benzidine was not detected by TLC.

## APPENDIX II

NAME AND COLOUR INDEX NUMBER OF SOME DIRECT DYES  
CONTAINING THE BENZIDINE MOIETY\*

Benzidine-Based Dye	C.I. No.	Benzidine-Based Dye	C.I. No.
1. Pyramidal Brown (LDC)	21060	55. Diazol Brown MA	22320
2. Congo GR(A)	22000	56. Direct Green 21:1	22322
3. Direct Yellow 24	22010	57. Direct Brown 60	22325
4. Diazo Violet R	22020	58. Triazol Red 6B	22330
5. Direct Brown 86	22030	59. Diphenyl Brown RN	22335
6. Diazo Brown R Extra	22035	60. Direct Brown 58	22340
7. Direct Brown 56	22040	61. Direct Brown 59	22345
8. Direct Brown 165	22045	62. Direct Red 88	22360
9. Direct Violet 88	22046	63. Direct Orange 1	22370
10. Diamine Brown S	22050	64. Direct Orange 1	22375
11. Pyramine Orange 3G	22060	65. Direct Orange 2	22380
12. Pyramine Orange RR	22070	66. Direct Orange 33	22385
13. Paranil Bordeaux B	22080	67. Alkali Yellow R	22390
14. Oxamine Scarlet B	22090	68. Wool Red G	22400
15. Oxamine Red B	22095	69. Direct Red 53	22405
16. Oxamine Orange G	22100	70. Direct Yellow 20	22410
17. Diazo Black R Extra	22110	71. Oxamine Red BN	22415
18. Direct Red 28	22120	72. Direct Red 59	22420
19. Glycine Red	22125	73. Direct Orange 1	22430
20. Direct Orange 8	22130	74. Direct Violet 43	22440
21. Direct Orange 25	22135	75. Direct Violet 3	22445
22. Direct Dye	22140	76. Direct Violet 42	22450
23. Direct Red 10	22145	77. Direct Blue 230	22455
24. Direct Red 17	22150	78. Direct Violet 27	22460
25. Direct Red 13	22155	79. Direct Violet 17	22465
26. Brilliant Congo G	22160	80. Direct Violet 36	22470
27. Direct Dye	22165	81. Direct Blue 16	22475
28. Direct Red 74	22170	82. Direct Violet 22	22480
29. Chlorazol Orange 2R	22175	83. Direct Blue 19	22485
30. Direct Red 42	22180	84. Direct Blue 58	22490
31. Direct Orange 101	22190	85. Naphthamine Blue 3R	22495
32. Acid Orange 45	22195	86. Direct Red 44	22500
33. Direct Red 60	22200	87. Direct Blue 42	22505
34. Direct Red 43	22205	88. Direct Violet 45	22510
35. Zambesi Brown GG	22210	89. Direct Violet 85	22520
36. Glycine corinth	22220	90. Alkali Dark Brown G, V Alkali Red Brown RR, 3R, T	22530
37. Para Green BBL	22230	91. Direct Blue 49	22540
38. Acid Red 323	22238	92. Direct Grey R	22545
39. Direct Red 37	22240	93. Direct Violet 12	22550
40. Acid Red 85	22245	94. Direct Violet 4	22555
41. Direct Yellow 1	22250	95. Direct Blue 48	22565
42. Cloth Orange	22255	96. Direct Violet 1	22570
43. Brilliant Direct Orange G	22260	97. Direct Black 29	22580
44. Mordant Dye-Cloth Brown R	22270	98. Naphthamine Black RE/ Naphthylamine Diazo Black	22585
45. Palatine Chrome Red RX	22275	99. Direct Blue 2	22590
46. Direct Red 18	22280	100. Direct Blue 64	22595
47. Cloth Brown G	22285	101. Diamine Nitrazol Green BB	22600
48. Direct Red 52	22290	102. Naphthamine Blue 2B	22605
49. Ozamine Maroon	22300	103. Direct Blue 6	22610
50. Direct Red 29	22305	104. Direct Black 15	22620
51. Direct Red 33	22306	105. Direct Blue 177	22625
52. Direct Red 1	22310	106. Direct Violet 38	22630
53. Direct Brown 2	22311		
54. Direct Green 60	22315		

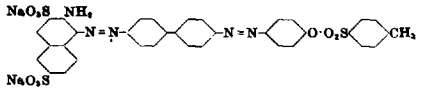
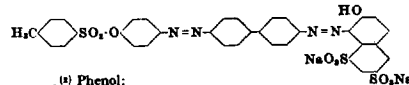
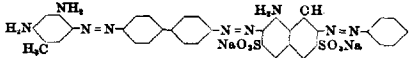
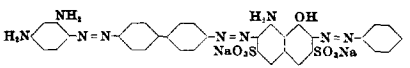
107.	Direct Dye	22640	171.	Direct Black 14	30345
108.	Direct Brown 7	30035	172.	Direct Blue 11	30350
109.	Direct Brown 171	30040	173.	Acid Black 70	30355
110.	Direct Brown 1	30045	174.	Direct Dye	30360
111.	Direct Brown 79	30050	175.	Direct Brown 151	31685
112.	Direct Brown 61	30055	176.	Direct Dye	31690
113.	Direct Brown 20	30060	177.	Direct Dye	31695
114.	Direct Dye	30065	178.	Direct Brown 24	31700
115.	Direct Brown 158	30070	179.	Direct Brown 57	31705
116.	Direct Dye	30075	180.	Direct Brown 51	31710
117.	Direct Dye	30080	181.	Direct Dye	31715
118.	Direct Dye	30085	182.	Direct Brown 62	31720
119.	Direct Blue 38	30090	183.	Direct Brown 27	31725
120.	Direct Dye	30095	184.	Direct Brown 26	31730
121.	Direct Brown 17	30100	185.	Direct Brown 54	31735
122.	Direct Dye	30105	186.	Direct Brown 10J	31740
123.	Direct Brown 1:2	30110	187.	Direct Dye	31745
124.	Direct Dye	30115	188.	Direct Brown 190	31750
125.	Direct Brown 154	30120	189.	Direct Brown 159	31755
126.	Direct Brown 68	30125	190.	Direct Black 40	31760
127.	Direct Dye	30130	191.	Direct Dye	31765
128.	Direct Brown 5	30135	192.	Direct Dye	31770
129.	Direct Brown 6	30140	193.	Direct Green 22	31775
130.	Direct Brown 95	30145	194.	Direct Dye	31780
131.	Direct Brown 175	30150	195.	Direct Brown 46	31785
132.	Direct Brown 21	30155	196.	Direct Green 21	31790
133.	Direct Dye	30160	197.	Direct Dye	31793
134.	Direct Brown 173	30165	198.	Direct Dye	31795
135.	Direct Dye	30170	199.	Direct Dye	31800
136.	Direct Dye	30175	200.	Direct Dye	31805
137.	Direct Dye	30180	201.	Direct Black 27	31810
138.	Direct Dye	30190	202.	Direct Dye	31815
139.	Direct Dye	30195	203.	Direct Dye	31820
140.	Direct Dye	30200	204.	Direct Dye	31825
141.	Direct Blue 43	30205	205.	Direct Dye	31830
142.	Direct Dye	30210	206.	Direct Dye	31835
143.	Direct Dye	30215	207.	Direct Dye	31840
144.	Direct Green 39	30220	208.	Direct Dye	31845
145.	Direct Green 58	30225	209.	Direct Black 83	31850
146.	Direct Dye	30230	210.	Direct Dye	31855
147.	Direct Black 38	30235	211.	Direct Brown	35060
148.	Direct Black 11	30240	212.	Direct Dye	35065
149.	Direct Black 4	30245	213.	Direct Dye	35070
150.	Direct Dye	30250	214.	Direct Black	35075
151.	Leather Dye	30255	215.	Direct Dye	35080
152.	Acid Black 69	30260	216.	Direct Blue 131	35085
153.	Direct Black 41	None	217.	Direct Dye	35240
154.	Direct Dye	30265	218.	Direct Dye	35400
155.	Direct Black 131	30270	219.	Direct Black 100	35415
156.	Acid Black 66	30275	220.	Direct Brown 33	35520
157.	Direct Green 1	30280	221.	Direct Brown 70	35530
158.	Direct Green 10	30285	222.	Direct Brown 73	35535
159.	Direct Green 12	30290	223.	Direct Dye	35650
160.	Direct Green 6	30295	224.	Direct Brown 31	35660
161.	Direct Dye	30300	225.	Direct Brown 43	35700
162.	Direct Green 19	30305	226.	Direct Brown 13	35710
163.	Direct Green 9	30310	227.	Direct Brown 14	35715
164.	Direct Green 8	30315	228.	Direct Brown 215	35720
165.	Direct Dye	30320	229.	Direct Dye	35900
166.	Direct Brown 75	30325	230.	Direct Brown 25	36030
167.	Direct Green 7	30330	231.	Direct Dye	36040
168.	Direct Dye	30335	232.	Direct Dye	36210
169.	Acid Black 94	30336	233.	Direct Brown 74	36300
170.	Direct Blue 51	30340	234.	Direct Brown 111	None
			235.	Direct Black 31	"
			236.	Resin F Black WP	"

\*Synonyms and trade names are listed in the Colour Index [1]

Adapted from references 1,43,(E Angstadt, written communication, January 1979)

APPENDIX III

BENZIDINE-BASED DYES REPORTED TO BE COMMERICALLY AVAILABLE IN THE UNITED STATES\*

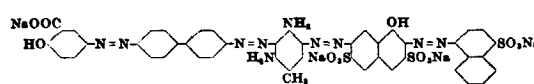
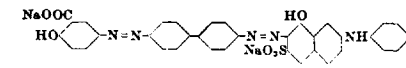
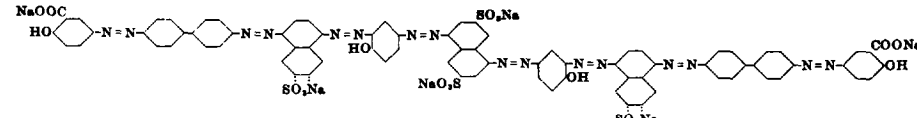
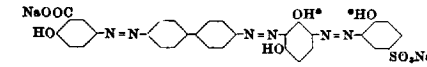
Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
 <p>3-Amino-2,7-naphthalenedisulfonic acid Benzidine Phenol; then esterify the hydroxy group with p-toluenesulfonyl chloride</p>	22195	2429-80-3	Not reported; less than 3 manufacturers	Not listed	Dyeing of cotton, silk, nylon, and leather; heavy metal salts used as pigments	Unknown
 <p>(1) Phenol; Benzidine (1) G acid then esterify the phenol hydroxy group with p-toluenesulfonyl chloride There are closely related dyes in which benzidine may be replaced by tolidine and other esterifying agents may be used. See C.I.23635 and C.I.24125</p>	22245	3567-65-5	67,000(1975) 22,245(1978)	2,190(1976) 1,000(1978)	Dyeing of cotton, wool, silk, nylon, and viscose; Viqueureux printing	525
 <p>(1) Toluene-2,4-diamine Benzidine (1) (acid) H acid (alk.) (s) ← Aniline</p> <p>Aqueous solution + HCl conc. — corinth ppt; NaOH conc. — greyish blue ppt.</p>	30245	2429-83-6	26,444(1978)	Not listed	Dyeing of cotton, wool, silk, nylon, leather, and paper	Unknown
 <p>(1) m-Phenylenediamine Benzidine (1) (acid) H acid (alk.) (s) ← Aniline</p>	30235	1937-37-7 RTECS No. JM7170000	3,760,000(1976) 823,000(1978)	70,753(1976) 49,525(1977) 170,442(1978)	Dyeing of leather, plastics, cotton, wool, and silk; aqueous inks, biological stain; wood flour used as a resin filler, wood stain; typewriter ribbons	13,072

APPENDIX III (CONTINUED)

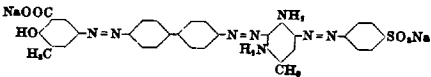
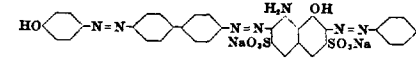
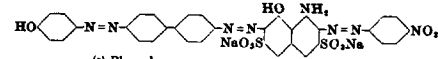
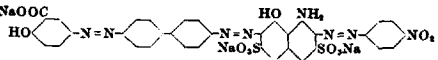
Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
<p>C.I. Direct Blue 2 (Dull Blue)</p> <p>Benzidine <sup>(a)</sup> (alk.) Gamma acid <sub>(b)</sub> (alk.) H acid</p>	22590	2429-73-4	218,435(1978)	38,478(1976) 30,755(1978)	Dyeing of cotton, leather, and paper	1,958
<p>C.I. Direct Blue 6 (Blue)</p> <p>Benzidine <math>\rightleftharpoons</math> (alk.) H acid (2 mol.)</p> <p>Aqueous solution + HCl conc. — navy blue, ppt; + NaOH conc. — dark violet, ppt.</p>	22610	2602-46-2 RTECS No. QJ6400000	327,000(1976) 61,524(1978)	4,409(1978)	Dyeing of leather, cotton, silk, paper; aqueous writing inks, biological stains	832
<p>C.I. Direct Brown 1 (Brown)</p> <p>Benzidine <sup>(a)</sup> Salicylic acid <sub>(b)</sub> [m-Phenylenediamine (acid) + Sulfanilic acid]</p>	30045	2586-58-5	Not listed	4,409(1978)	Dyeing of leather, paper, silk, nylon, wool and cotton	Unknown
<p>C.I. Direct Brown 2 (Reddish brown)</p> <p>Benzidine <sup>(a)</sup> Salicylic acid <sub>(b)</sub> (alk.) Gamma acid</p>	22311	2429-82-5	125,000(1975) 27,725(1978)	18,739(1976) 2,205(1977)	Dyeing of leather, paper, silk, nylon, wool, and cotton; heavy metal salts used as pigments	106
<p>C.I. Direct Brown 6 (Brown)</p> <p>Benzidine <sup>(a)</sup> Salicylic acid <sub>(b)</sub> Resorcinol (b) + Sulfanilic acid</p> <p>This sequence of operations, which is that recorded for Congo Brown G, differs slightly from C.I. 1st Edition 598</p>	30140	NA	8,563(1978)	Not listed	Dyeing of leather, paper, silk, wool, and cotton	Unknown



APPENDIX III (CONTINUED)

Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
<p>C.I. Direct Brown 31 (Reddish brown) 35660 2429-81-4 37,406(1978) Not listed</p>  <p> <sup>(1)</sup> Salicylic acid  Benzidine <sup>(2)</sup> (Toluene-2,4-diamine (acid) → O-Phenylsulfonyl 2R acid);  then hydrolyze the benzenesulfonic ester group  <sup>(3)</sup> ← Naphthionic acid  (In some brands 2R acid is used instead of its O-phenylsulfonyl derivative* as in C.I.35650) </p>					Dyeing of leather and paper; heavy metal salts used as pigments; printing on cellulose (concentrated dye only)	Unknown
<p>C.I. Direct Brown 59 (Blackish brown) 22345 NA Not listed "</p>  <p> <sup>(1)</sup> Salicylic acid  Benzidine <sup>(2)</sup> (alk) N-Phenyl Gamma acid </p>					Dyeing of cotton, wool, and silk; leather; occasional use on chrome and vegetable tannages	"
<p>C.I. Direct Brown 74 (Brown) 36300 NA 32,414(1978) "</p>  <p> <sup>(1)</sup> Salicylic acid  Benzidine <sup>(2)</sup> [1,6(and 1,7)-Cleve's acid] (2 mol.) → <sup>(3)</sup> [Pheno (2 mol.) ± 4,8-Diamino-2,6-naphthalenedisulfonic acid] </p> <p>HNO<sub>3</sub> conc. — dull red solution, turns yellow brown  Aqueous solution + HCl conc. — brownish yellow to olive ppt;  + NaOH conc. — orange brown</p>					Dyeing of cotton, wool, silk, leather, chrome tannage (occasional)	"
<p>C.I. Direct Brown 95 (Reddish brown) 30145 16071-86-6 346,000(1975) 8,205(1976)</p> <p>RTECS No. 595,000(1976) 15,962(1977)  JM78780000 75,953(1978) 5,512(1978)</p> <p>Copper complex derived from</p>  <p> <sup>(1)</sup> Salicylic acid  Benzidine <sup>(2)</sup> [Copper complex formed at * from 2-Amino-1-phenol-4-sulfonic acid → Resorcinol] </p> <p>In Sirius Supra Brown BRLN 20% of the salicylic acid is replaced by 2,3-cresotic acid</p>					Dyeing of cotton, wool, silk paper, plastics, and leather; heavy metal salts used as pigments	714

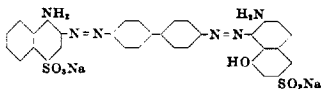
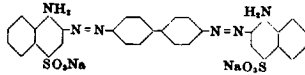
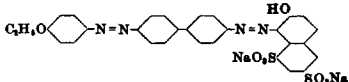
APPENDIX III (CONTINUED)

Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
C.I. Direct Brown 111 (Reddish brown) Structure Unknown	No C.I. No.	NA	Not listed	Not listed	Dyeing of cotton and leather; chrome tannage (occasional)	Unknown
C.I. Direct Brown 154 (Brown)	30120	6360-54-9	63,816(1978)	"	Dyeing of cotton, wool, silk, leather, and paper; direct printing on cellulosic weave and silk fabrics	322
 <p>Benzidine <math>\leftarrow</math> 2,3-Cresotic acid [Toluene-2,4-diamine <math>\leftarrow</math> Sulfanilic acid]</p>						
C.I. Direct Green 1 (Dull green)	30280	3626-28-6	57,000(1974) 12,666(1978)	"	Dyeing of cotton, wool, silk, nylon, leather, and paper; aqueous inks; direct printing on cellulosic, silk, and nylon fabrics	1,850
 <p>Benzidine <math>\leftarrow</math> (a) Phenol (1) (acid) H acid (alk.) (1) <math>\leftarrow</math> Aniline</p>						
C.I. Direct Green 6 (Dull green)	30295	4335-09-5	143,000(1974) 109,076(1978)	4,659(1978)	Dyeing of cotton, wool, silk, and nylon; aqueous inks, pigments, leather, paper, and soap; direct printing on nylon	1,095
 <p>Benzidine <math>\leftarrow</math> (a) Phenol (1) (alk.) [H-acid (acid) <math>\leftarrow</math> p-Nitroaniline]</p> <p>Aqueous solution + HCl conc. — dull bluish green ppt; + H<sub>2</sub>SO<sub>4</sub> (10%) — bluer; + NaOH conc. — dark green to olive</p>						
C.I. Direct Green 8 (Dull green)	30315	5422-17-3	Not reported; less than 3 manufacturers	250(1977)	Dyeing of cotton, wool, silk, nylon, leather, and paper	Unknown
 <p>Benzidine <math>\leftarrow</math> (a) Salicylic acid (a) (alk.) [H acid (acid) <math>\leftarrow</math> p-Nitroaniline]</p>						

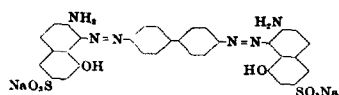
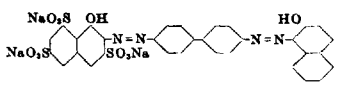
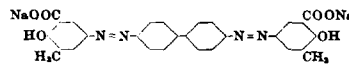
APPENDIX III (CONTINUED)

Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
<p>C.I. Direct Orange 1 (Yellowish orange)***</p> <p> <chem>NaOOCc1ccc(O)cc1=N=Nc2ccc(cc2)N=Nc3c(C(=O)O)nc(C4=CC=CC=C4S(=O)(=O)[O-])n3</chem> </p> <p>           Benzidine <math>\swarrow</math> Salicylic acid  <math>\searrow</math> 3-Carboxy-1-(p-sulphophenyl)-5-pyrazolone         </p>	22370	6459-87-6	Not reported; less than 3 manufacturers	Not listed	Dyeing of cotton, wool, silk, nylon, paper, and leather direct printing on cellulose and nylon	Unknown
<p>C.I. Direct Orange 8 (Reddish orange)</p> <p> <chem>Nc1ccc(S(=O)(=O)[O-])cc1=N=Nc2ccc(cc2)N=Nc3ccc(O)cc3</chem> </p> <p>           Benzidine <math>\swarrow</math> (1) Naphthionic acid  <math>\searrow</math> (1) Salicylic acid         </p> <p><i>In some brands part of the salicylic acid is replaced by 2,3-cresotic acid (C.I.22140) and part of the naphthionic acid by other aminonaphthalene-sulfonic acids (C.I.22165)</i></p>	22130	2429-79-0	86,000(1976) 27,208(1978)	4,066(1976)	Dyeing of cotton, wool, silk, nylon, and paper	"
<p>C.I. Direct Red 1 (Bluish red)</p> <p> <chem>NaOOCc1ccc(O)cc1=N=Nc2ccc(cc2)N=Nc3c(N)ccc(S(=O)(=O)[O-])c3</chem> </p> <p>           Benzidine <math>\swarrow</math> (1) Salicylic acid  <math>\searrow</math> (2) (acid) Gamma acid         </p>	22310	2429-84-7	132,000(1975) 26,370(1978)	4,409(1977)	Dyeing of cotton, wool, silk, nylon, paper, and leather	55,508
<p>C.I. Direct Red 10 (Bordeaux)</p> <p> <chem>Nc1ccc(S(=O)(=O)[O-])cc1=N=Nc2ccc(cc2)N=Nc3c(O)ccc(O[Na])c3</chem> </p> <p>           Benzidine <math>\swarrow</math> (1) Naphthionic acid  <math>\searrow</math> (2) Nevile and Winther's acid         </p>	22145	2429-70-1	Not reported; less than 3 manufacturers	100(1975)	Dyeing of cotton, wool, silk, and leather; biological stain	Unknown

APPENDIX III (CONTINUED)

Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
<p>C.I. Direct Red 13 (Bordeaux)</p>  <p>Benzidine <sup>(1)</sup> Naphthionic acid  <sup>(2)</sup> (acid) Gamma acid            (In Diamine Bordeaux N (B)) part of the Gamma acid was replaced by J acid)</p>	22155	1937-35-5	Not reported; less than 3 manufacturers	Not listed	Dyeing of cotton, wool, nylon, paper, and leather (chrome tannage); printing of cellulose	1,640
<p>C.I. Direct Red 28 (Yellowish red)</p> <p>Classical name Congo Red</p>  <p>Benzidine ⇌ Naphthionic acid (2 mol.)</p> <p>Soluble in water (yellowish red) and ethanol (orange); very slightly soluble in acetone  <math>H_2SO_4</math> conc. — deep blue; on dilution — paler blue, blue ppt.            Aqueous solution + HCl conc. — reddish blue ppt.; + Acetic acid — bluish violet, then reddish blue ppt.; + NaOH conc. — yellow</p>	22120	573058-0 RTECS No. QK1400000	37,327(1978)	11,000(1974) 33,069(1978)	Dyeing of cotton, wool, silk, and paper; biological stain and indicator; (first synthetic direct cellulose dye)	523
<p>C.I. Direct Red 37 (Red)</p>  <p>Benzidine <sup>(1)</sup> Phenol;  <sup>(2)</sup> G acid            then ethylate the phenol hydroxy group by heating under pressure with ethyl chloride in aqueous ethanol solution in the presence of sodium carbonate</p>	22240	3530-19-6	63,000(1975)	Not listed	Dyeing of cotton, wool, silk, leather and paper; direct and discharge printing of cellulose and nylon	1,052

APPENDIX III (CONTINUED)

Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
 <p>Benzidine <math>\rightleftharpoons</math> (acid) Gamma acid (2 mol.)</p>	22570	2586-60-9	Not reported; Not listed less than 3 manufacturers		Dyeing of cotton, wool, silk, leather, and paper; biological stain	Unknown
 <p>Benzidine <math>\rightleftharpoons</math> 1-Naphthol-3,6,8-trisulfonic acid 2-Naphthol</p>	22480	6426-67-1	" manufacturers	"	Dyeing of cotton, wool, silk, nylon, leather	"
 <p>Benzidine <math>\rightleftharpoons</math> 2,3-Cresotic acid (2 mol.)</p> <p>Aqueous solution + HCl conc. — brownish yellow, ppt; + NaOH conc. — reddish yellow, ppt.</p>	22410	6426-62-6	Imported only	3,900(1977)	Dyeing of cotton, silk, wool, nylon, leather	"
Resin Fast Black WP	No C.I.#	NA	84,620(1978)	Not listed	Dyeing of textiles, especially those subsequently finished with resins	"

\*This table lists the benzidine-based dyes that were reported as being commercially available by DETO [46] and reported as produced or imported by the US International Trade Commission (ITC) [3,42] or those to which potential exposure was found [44]. If less than three manufacturers make a dye, ITC does not publish the production figures.

\*\*A discussion of limitations of the estimation of worker exposure is contained in reference 44.

\*\*\*This dye may also be synthesized with cresotic acid in place of salicylic acid. The Colour Index designates both dyes as Direct Orange 1.

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