

ETAD



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Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry
U.S. OPERATING COMMITTEE OF ETAD

Contains No GSI

April 30, 1987



ETAD COMMENTS ON
DYNAMAC CORPORATION'S INFORMATION REVIEW
OF
SECONDARY AMINOANTHRAQUINONE DYES
(1R-486, JULY 31, 1986, WORKING DRAFT)

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These comments are submitted on behalf of the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry (ETAD), an international organization formed in 1974 to address health and environmental matters relating to organic colorants. These comments were prepared by the U.S. Operating Committee of ETAD, which consists of representatives of the 10 U.S. members of ETAD (see Appendix I).

We believe that the Information Review provides a useful overview of the relevant information regarding amino anthraquinone dyes. However, the accuracy of the Information Review would be improved if revised to reflect the following comments.

For convenience the comments are given in the order of the text of the Information Review.

page (iii) Production and Use

Recent production data have been submitted to the EPA under the recent reporting requirements for partial updating of TSCA Inventory Data Base.

It should be noted that only the Acid, Basic, and Disperse dyes included in the list are used for dyeing fibers. The Solvent dyes are used for the coloration of plastics, smokes, and a variety of minor uses.

Environmental Release and Concentrations

There is negligible release of Solvent Green 3 and Solvent Violet 13 to the environment in aqueous waste streams. In the case of the other listed dyes the primary release is in aqueous streams and solid waste.

Fugitive dust emissions from both manufacturing and processing plants are a negligible source of environmental release for dyes, as emission sources are generally scrubbed.

page (iv) Environmental Fate

This section should be expanded to reflect the published studies on the adsorption of dyes on activated sludge (in the cited reference Hitz, Huber and Reed, 1978, and also several reports by G. Shaul et al, EPA Cincinnati). Of particular note is the high degree to which Basic dyes adsorb on sludge.

Although the report by Horning (1978) does not include any of the listed secondary aminoanthraquinone dyes, it does provide data on the effectiveness of various dye-bath effluent treatment processes which are relevant to the evaluation of likely environmental fate of these substances.

Solvent dyes are unlikely to reach aqueous environments in significant amounts which could raise any concern about bioaccumulation.

Additional information on two listed dyes (Disperse Blue 7 and Acid Blue 25) and on a further secondary aminoanthraquinone dye (Acid Blue 40) is included in a report by W.C. Tincher, "Survey of the Coosa Basin for Organic Contaminants from Carpet Processing", October 1973 (Appendix II).

page (iv) Environmental Effects

It would be appropriate to provide some comparison here between observed effect levels and observed or likely environmental concentrations.

page (iv) Summary of Environmental Hazard Potential

line 2

As the selection of the 10 aminoanthraquinone dyes is somewhat arbitrary it would be better to avoid such unspecific statements as "fairly low", and to relate specific effect levels to observed environmental concentrations.

page 1 Chemical and Physical Information

D Chemical Properties

Line 1 Anthraquinone dyes are probably the least sensitive of commercial dyes to U.V. radiation. In terms of chemical stability the aminoanthraquinones are much more stable to oxidizing agents than most aromatic amines.

Solvent Green 3 exemplifies the property of high thermal and oxidative stability which is characteristic of anthraquinone dyes.

page 7 Exposure Data

para. 1 The U.S. International Trade Commission report for 1985 includes specific data for only Disperse Blue 3 of the ten selected dyes.

Disperse Blue 3	1985 sales	436,000 lb.
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para. 5 The AATCC Guide designates suppliers not manufacturers of specific dyes.

para. 6 The manufacturing route information is outdated and is not relevant to the review of the environmental effects of the derived products. This section should be deleted.

page 11 B. Use Table 5

Acid Blue 25 is not used to dye acetate. Acid Blue 25 is used to dye anodized aluminum but not aluminum. Its use for dyeing leather and soap is stated twice by mistake. Acid Green 25 is not a paper dye. Basic Blue 21 use for dyeing polyester is limited to basic dyeable polyester. Disperse Blue 3 and Disperse Blue 7 are not normally used to dye wool.

page 12 Environmental Information

Nowadays dyes contained in the effluents from the dyeing process are either treated in a site effluent treatment plant or are discharged to a publically owned treatment works (POTW). There is no direct release to the environment of aqueous effluents from the dyeing process. The release of dyes from dyed textiles in landfills has not been demonstrated and is unlikely.

The estimations of the amount of various dyes released from dyeing processes need to be modified to take into account the more recent changes in dyeing technology which have effected a substantial reduction in the amounts released. Examples of these which largely eliminate environmental releases are:

- o continuous dyeing of polyester
- o continuous dyeing of carpets with Acid dyes
- o dyeing of carpets
- o Kuester dyeing

page 13 para. 1

This paragraph should be modified to clarify that the reported dye concentrations relate to formulated dye and not active dye ingredients, and also that the data do not relate specifically to the substances under review.

page 13 B. Environmental Fate

Relatively small amounts of dyes are released to the open environment and these tend to be removed from the water body through adsorption processes (on to sediments and plants).

page 15 4 Biodegradation

The publication by Paggi and Brown (1986), attached as Appendix III, provides additional information on the aerobic biodegradation of several anthraquinone dyes, including Basic Blue 22.

page 16 Summary

The conclusion of high bioaccumulation potential for the listed substances is speculative, and not supported by any of the cited references. Bioaccumulation of the water-soluble Acid and Basic dyes is most unlikely.

page 18 Environmental Effects

The effects of two secondary aminoanthraquinones (Acid Blue 25 and Disperse Blue 26) on plant growth (Sorghum bicolor, Helianthus annuus and Glycine max) have been studied at concentrations of 1, 10, 100 and 1000 mg dye/kg dry soil. Under practical conditions the level of any one dye reaching

agricultural land through contaminated sludge is unlikely to exceed 1 mg/kg (based on conservative assumptions). There was no significant effect on germination and emergence of any of the levels tested, and no significant effect on plant growth at the lower three levels. At 1000 mg/kg significant reduction of growth was observed in Sorghum and Helianthus, and marginal reduction in Glycine max. (See Appendix IV)

page 19

Table 6

Basic Blue 22	rainbow trout	LC 50 (48 hour) > 500 mg/l
Disperse Blue 7	rainbow trout	LC 50 (48 hour) 10-100 mg/l

Respectfully submitted,



Eric A. Clarke
Executive Secretary

APPENDIX I

U.S. MEMBER COMPANIES OF ETAD

American Hoechst Corporation
Atlantic Industries, Incorporated
BASF Corporation
Carey Industries, Incorporated
Ciba-Geigy Corporation
Crompton & Knowles Corporation
ICI Americas, Incorporated
Mobay Corporation
Morton Thiokol, Incorporated
Sandoz Chemicals Corporation

Final Report

**Survey of the Coosa Basin for Organic
Contaminants from Carpet Processing**

by

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Prepared Under Contract No.

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for

**Environmental Protection Division
Department of Natural Resources
State of Georgia**

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Act Amendments of 1972.

October, 1978

I. Introduction

The carpet industry uses and discharges approximately 20 billion gallons of water annually [1]. This is of the order of 14 gallons of water per pound of carpet produced [2]. Over 50% of the total or 7 billion gallons of water is discharged each year to the Coosa River basin [3,4]. Data on the carpet industry in Georgia is shown in Table 1.

Most of the water used in carpet manufacturing is used in the dyeing process. Carpet dyeing and rinse water contains substantial quantities of dissolved and suspended organic and inorganic compounds. Since the Coosa River and its tributaries serve as a source of water for a number of cities and towns, the presence of these compounds in carpet wastewater is of considerable concern to officials responsible for water quality in the states of Georgia and Alabama.

A previous study [5,6] has indicated that carpet dyeing and finishing wastes may be classified in five major groups. These groups and the approximate quantities of each type discharged annually by the carpet industry are:

- Inorganic compounds--13 million pounds
- Volatile organic compounds-- 40 million pounds
- Dyes --0.3 million pounds
- Surfactants --16 million pounds
- Other organic compounds --12 million pounds

Although dyes constitute a small portion of the total amount of waste discharged in carpet processing, this group is very important for several reasons. First, analytical procedures for the determination of individual dyes in complex mixtures are not available. Procedures for inorganic

Table 1

Distribution of the Georgia Carpet Industry
(SIC CODE 227)

<u>Area</u>	<u>No. of Plants</u>	<u>Value of Shipments (Million Dollars)</u>	<u>%</u>
United States (Total)	-	2,937	100.00
State of Georgia	248	1,727	58.8
Whitfield County	128	738	25.1
Gordon County	36	175	6.0
Bartow County	21	147	5.0
Murray County	19	36	1.2
Gilmer County	5	21	0.7
Eight Northwest Georgia Counties +*	-	1,499	51.0

+ Whitfield, Bartow, Catoosa, Chattooga, Floyd,
Gordon, Murray, Walker.

* Data from Georgia Economic Model which may be on
slightly different basis than other data in the
table [6].

compounds [7], for volatile organic compounds [8], and for surfactants [9] in wastewater are generally available. Extensive work on the analytical chemistry of dyes [10] has been reported in the literature but no analytical system has been developed for determination of dyes at the fractional parts-per-million level in the complex mixtures typically encountered in waste streams.

Second, dyes in textile waste streams are important because they are not readily removed by typical waste treatment processes. Most textile wastes receive some type of biological oxidation (lagoon, oxidation pond, trickling filter, activated sludge) prior to discharge to streams and rivers. Although biological treatment is effective in reduction of biochemical oxygen demand, it is not effective in color reduction. For example, in a joint Environmental Protection Agency - American Dye Manufacturers Institute study of 9 dyes [11], laboratory biological treatment removed from 42% to 97% of the color when the dyes were added to domestic waste and treated. Only 1 acid and 1 disperse dye were used in this study and both showed less than 50% removal. Removal of color from 30% [12] to 68% [13] has been reported for activated sludge treatment plants with substantial textile dye wastewater in the influent. These data clearly demonstrate the resistance of dyes to treatment techniques currently employed.

Third, the possible long term health effects of dyes and dye degradation products are becoming of increasing concern. The possible mutagenic, carcinogenic, and/or teratogenic effects of dyes are now under investigation in a number of laboratories [14-16]. The preliminary results from this work suggest that analysis for specific dyes may be important in identifying and quantifying possible health hazards.

In addition to quantitative determination of dyes in wastewater and in streams and rivers, an analytic system for dyes is important for evaluation of the efficiency of dye removal by both current and new treatment systems. Thus, the work was undertaken to develop an analytical system to determine the concentration of dyes commonly used in carpet processing.

The work is divided into four parts. Part I describes the analytical procedures developed for acid and disperse dyes. Part II describes the use of these procedures for analysis of samples collected in the Coosa River basin, Part III details the use of the system for evaluating advanced treatment systems for dye removal efficiencies, and Part IV describes the extension of the analytical system to direct dyes.

Part I
Development of Analytical Procedures
for Dyes in Wastewater

A. Objective and Approach

The objective of the first phase of the project was the development of an analytical system for determination of the concentrations of acid and disperse dyes in wastewater at the parts-per-billion level. Based on the previous studies of chemical use in the carpet industry [5], it was estimated that dyes would be present in carpet processing wastes at the fractional parts-per-million level. Analytical techniques for dyes (visible absorption spectroscopy) can readily detect dyes at the parts-per-million level. An approximate 100-fold concentration procedure was necessary therefore, to obtain dyes at a concentration suitable for quantitative analysis. Since dyes always occur in mixtures in wastewater, a separation procedure was also required. The most distinctive characteristic of dyes is their very strong absorption of energy in the visible region of the spectrum. This characteristic was selected for development of a method for quantitation of the concentrated and separated dyes.

B. Dyes Selected for Study

Two major classes of dyes - acid dyes and disperse dyes - and a few basic and direct dyes are used in carpet piece dyeing. Fifteen dyes from the acid and disperse classes were selected for study. These dyes are shown in Table 2. These 15 dyes account for over 50% of all the dyes used in carpet piece dyeing [5]. The dyes from this group whose structures are known are shown in Appendix A.

Table 2

Dyes Selected for Development
of Analytical Techniques

Acid Yellow 19

Acid Yellow 135

Acid Yellow 151

Acid Orange 128

Acid Red 151

Acid Red 337

Acid Blue 25

Acid Blue 40

Disperse Blue 7

Disperse Blue 120

Disperse Yellow 3

Disperse Yellow 23

Disperse Yellow 54

Disperse Red 55

Disperse Red 60

0 0 1 6

C. Preparation of Dye Standards

Dyes as sold by dyestuff manufacturers contain many materials in addition to the dye component. Commercial disperse dyes usually contain from 15 to 30% dye and commercial acid dyes are generally of the order of 50% pure [5]. Materials present in commercial dyes in addition to the dye include dispersing agents, salt, sugar, sodium sulfate, and other additives and diluents. For the purposes of this study it was necessary to prepare samples of pure dye to use as analytical standards.

Disperse dyes were purified by Soxhlet extraction with benzene. Approximately 50 grams of dye were placed in a glass extraction thimble and extracted with benzene for 12 hours. The dye is soluble in benzene but the more polar diluents and dispersing agents are insoluble. The dye was recovered by removal of the benzene in a rotary evaporator. The recovered dye was again extracted with benzene and the process repeated until the extinction coefficient of the dye reached a constant value. This usually occurred after three extraction and recovery cycles.

Acid dyes were purified by recrystallization from methanol. Approximately 50 grams of dye were dissolved in 500 milliliters of methanol. The acid dyes are readily soluble in methanol but the inorganic salts present in the commercial dye are insoluble. The solution was filtered and the dye recrystallized from the filtrate. This procedure was also repeated until the dye extinction coefficient reached a constant value. This also generally required about three cycles.

Standard solutions were prepared by weighing out 1.00000 grams of dye on a Mettler Microanalytical Balance (capable of weighing to 0.000001 grams).

The dye was transferred to a 1 liter volumetric flask and solvent added to give 1 liter of solution.

Some difficulty was encountered in dissolving the pure disperse dyes in benzene, the selected solvent. With no dispersing agent present the dye was difficult to dissolve completely. This problem was solved by pasting the dye with dimethylformamide and then adding the benzene. Thus the standard disperse dye solution contained the dye dissolved in a 1% dimethylformamide (DMF), 99% benzene solvent. Similarly, the standard acid dye solutions were prepared in 1% DMF, 99% methanol solvent.

Solutions containing 5, 10, and 20 parts-per-million (ppm) of the pure dyes in solution were prepared by dilution of the standard 1 gram per liter solution with 1% DMF in either methanol or benzene.

D. Concentration Procedures

The necessity for an approximately 100-fold increase in dye concentration suggested that some type of adsorption procedure would be most convenient for the concentration step. Previous work [6,17-20] on a number of possible adsorbents had shown that macroreticular resins were promising for removal of dyes from solution. These resins are copolymers of styrene with divinylbenzene and have a high surface-to-volume ratio. Of the available resins Amberlite XAD-2 (Rohm and Haas Company) was found to give the best removal and recovery of dyes from wastewater. Further work, therefore, was concentrated on development of a recovery and concentration procedure based on XAD-2 resin adsorption.

One problem with adsorption of disperse dyes arises due to the fact that these dyes are not molecularly dispersed in water. They are, therefore,

not efficiently removed by adsorption processes. During the course of this work it was discovered that the addition of 10% DMF to water dispersion of disperse dyes would greatly increase the recovery of the dyes from the water.

1. Column Preparation

Lab-Crest 9 μ by 500 μ chromatography columns equipped with demountable Teflon stopcocks were used for column preparation. Amberlite XAD-2 resin (30 ml) was slurred in approximately 60 ml of methanol and run through the column as rapidly as possible (\sim 5 minutes). A glass wool plug was used in the bottom of the column to retain the resin. The column was then washed with 40 ml of benzene at approximately 2 bed volumes per hour. The benzene wash was followed by a 40 ml methanol upflow wash at 8 bed volumes per hour to reclassify the column and to remove air bubbles resulting from the benzene wash. The column was then washed with 40 ml of each solvent used in the extraction of dye from the resin to ensure that any impurities likely to come off the column with the dyes would be removed. The column was given a final wash with 200 ml of distilled water and stored under distilled water until used. Column preparation and use procedures have been described in detail in Rohm and Haas Technical Bulletins [17,21].

2. Recovery Studies

In preliminary development of the recovery system, 200 ml of a 1 ppm dye solution in 10% DMF and 90% water were passed through the column at 8 bed volumes per hour (approximately 350 ml/hour).

The column was then washed with 50 ml of 10% DMF and 90% water, the column inverted and back washed with selected solvents to remove the dyes. The removed dyes were taken up in a known volume of solvent and the dye concentration determined spectrophotometrically. The percent recovery of the dye could thus be determined from the known quantity of dye in the original solution and the amount recovered from the column by the back-washing procedure.

A number of experimental parameters were studied to establish details of the concentration procedure. The effect of column diameter was investigated using 9 mm and 15 mm columns. The results indicated that column diameter did not influence the recovery. Subsequent studies were carried out on the smaller columns to reduce the volume of recovery solvents required. Resin bed depths of 25 and 40 cm were studied also. Recoveries of dyes were slightly better at 40 cm so the larger bed depths were used in further studies.

Solvents used in recovery had a major effect on recovery efficiencies. Early studies showed that benzene readily removes disperse dyes from the resin column. Recoveries were better than 70% for each of the seven disperse dyes investigated. Recovery of acid dyes proved more difficult. A number of solvents including, DMF, methanol, tetrahydrofuran, pyridine and ammonia were tried. The system that gave best results involved back-washing with two solvent systems. The acid dyes were removed by first eluting with 40 ml of methanol followed by 40 ml of a pyridine, tetrahydrofuran, 1% ammonium hydroxide (40:40:20) solvent mixture, both at a rate of 4 bed volumes per hour. This elution system gave better than 70% recovery of each of the acid dyes.

3. Concentration system.

The final system developed for concentration of the dyes required the following steps. Columns were prepared as described previously. A 1000 ml Kelly infusion jar was attached to the top of the resin column with a 1 inch piece of silicone tubing of 0.1925 inches I.D. (Cole-Palmer). Wastewater (900 ml) and dimethylformamide (100 ml) were placed in the Kelly infusion jar and allowed to flow down the column at 4 bed volumes (120 ml) per hour. As indicated earlier, the dimethylformamide is added to increase the solubility of disperse dyes so that they will be adsorbed by the resin. If dye is present in the wastewater, colored bands will appear near the top of the column. After all the wastewater has passed through the column, the reservoir and column were washed with 50 ml of a 90% water- 10% dimethylformamide mixture to insure that all of the wastewater sample had contacted the column.

Since the dye is concentrated near the top of the column, the reservoir and stopcocks were removed and the column inverted for the subsequent elution steps. A 9 mm x 500 mm extension column was attached to the top to serve as a solvent reservoir. The column was first eluted with 40 ml benzene at approximately 2 bed volumes per hour. The disperse dyes are removed by benzene and the acid dyes remain on the column. The acid dyes were removed by first eluting with 40 ml of methanol followed by 40 ml of the pyridine, tetrahydrofuran, 1% ammonium hydroxide (40:40:20) mixture, both at a rate of 4 bed volumes per hour.

The benzene containing the disperse dyes was collected in a 50 ml round bottom flask equipped with a 24/40 ground glass joint. The flask was placed on a Buchi Rotavap K and benzene removed under aspirator vacuum at temperatures up to 100°C. The disperse dyes were taken up in a 1% dimethylformamide - 99% benzene solvent and made up to 10, 25 or 50 ml in a volumetric flask depending on the desired increase in concentration.

The methanol and solvent mixture extracts were combined in a 250 ml round bottom flask and rotavapped as described above for the benzene extracts. The residual acid dyes were taken up in 1% dimethylformamide - 99% methanol and made up to the desired volume.

The recovery efficiencies for 1 part-per-million solutions of the 15 important carpet dyes using the above procedure are shown in Table 3.

The concentration procedure was generally used to concentrate the dyes in 900 ml of wastewater in a volume of 10 ml for an overall concentration factor of 90. In some cases 2 columns were used and 900 ml of wastewater run through each column. Combining the eluents from the two columns gave a concentration factor of 180.

In addition to concentration of the dyes, the resin adsorption procedure also provided a separation of the acid and disperse classes of dyes. This separation was quantitative and greatly simplified the subsequent analytical procedures. The separated and concentrated acid and disperse dyes were now ready for the separation and quantitation procedure.

E. Separation and Quantitation

Dye mixtures have traditionally been separated by thin-layer chromatography. In previous work [6] on the 7 disperse dyes and 8 acid dyes

Table 3

Recovery of Carpet Dyes From Wastewater
By Resin Adsorption

<u>Acid Dyes</u>		<u>Disperse Dyes</u>	
Acid Yellow 151	75%	Disperse Yellow 23	77%
Acid Red 337	75%	Disperse Yellow 3	98%
Acid Yellow 19	70%	Disperse Yellow 54	77%
Acid Yellow 135	100%	Disperse Red 60	89%
Acid Orange 128	76%	Disperse Red 55	95%
Acid Red 151	97%	Disperse Blue 7	73%
Acid Blue 25	80%	Disperse Blue 120	83%
Acid Blue 40	84%		

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under investigation in this study it had been demonstrated that thin-layer chromatography could be used to separate both the acid and disperse dyes. This technique however, is difficult, does not lend itself to quantitative analysis, and is not readily adaptable to analyses of large numbers of routine samples.

Recent developments in technology have made rapid and quantitative separation of complex mixtures possible by high pressure liquid chromatography (HPLC). In this technique, components to be separated are dissolved in a suitable solvent and pumped onto a column containing a small particle, high surface area adsorbant. The differing partition of the various components between the stationary phase and the moving solvent phase results in a separation of the components as they move down the column. The instrument may be operated in a variety of modes depending on the nature of the stationary phase. In the case of small particle silica columns, a type of adsorption chromatography is responsible for the separation. In other cases a liquid-like hydrocarbon (e.g., C₁₈ chains) are adsorbed on or bonded to the column and a type of liquid-liquid partition is achieved. Ionic species may also be bonded to the column for separation of polar components. Columns containing specific pore sizes are available to obtain separations based on molecular size (exclusion chromatography). This range in operation mode combined with the variety of solvents and solvent mixtures which may be used as mobile phases provides an extremely versatile separation tool. Detection of components exiting from the column is usually achieved by adsorption in the UV and/or visible spectrum. Thus, the detector systems are especially sensitive to dye molecules and the aromatic portions of many

surfactant molecules. By operating in the visible region of the spectrum dyes can be detected and quantitated in the presence of the many other organic compounds in dye wastewater which do not absorb strongly in the visible region. Similarly, selection of absorbing wavelength makes possible determination of, for example, yellow dyes even when blue dyes elute at the same retention time. Thus wavelength selection can greatly simplify the separation process.

HPLC has been applied to the separation of both azo and anthraquinone dyes by a variety of techniques in a number of recent papers. An excellent review of this work had been presented by Pata [10]. HPLC was selected, therefore, as the method for separation and quantitation of the dyes selected for study in this work.

1. Equipment

Two different liquid chromatographs were used during the course of the work. Early development work was carried out on a Micromeritics Model 7000 HPLC. This instrument can be operated in either an isocratic (fixed solvent composition) or gradient (time variant solvent composition) mode. It is equipped with a variable wavelength UV-visible detector. The only difficulty encountered with this instrument was in analyses for blue dyes. The output of the detector light source was very low at wavelengths above 600 nm. It was not possible to detect blue dyes at low concentration.

Most of the later work employed a HPLC system assembled from components. This system had a Laboratory Data Control Pumping System (Constametric I and VI pumps with a Constametric II G Control Module and Model 7120 Gradient

Master), a Rheodyne Model 7120 Syringe Loading Sample Injector, a Tracor Model 970 Variable Wavelength Absorbance Detector, and a Houston Instrument Omniscrite Recorder with a built-in integrator. This instrument system performed very well in all dye analyses.

2. Analysis for Disperse Dyes

a. Column and Solvent Selection

The structure and polarity of disperse dyes suggested that absorption liquid chromatography would be the best technique for separation. In this technique, compounds are adsorbed on a polar surface and separations are achieved based on differences in polarity of components. Initially, a small particle 10 μ silica column was investigated for separation of disperse dyes. The disperse dyes were so strongly adsorbed on this column that they were very difficult to remove with even the most polar solvents.

Best separation of the disperse dyes was obtained with a column of intermediate polarity. The column employed was a 25 cm Partisil-10 PAC column (Whatman) which has cyanoethyl groups bonded to a 10 μ irregular silica substrate.

A solvent system consisting of a nonpolar component (nonsolvent) and a polar component (solvent) is generally used in adsorption chromatography as the mobile phase. Several polar compounds--tetrahydrofuran, dioxane, isopropyl alcohol, methyl alcohol, dimethylformamide--which are solvents for disperse dyes were considered for the strong solvent. Isooctane, cyclohexane,

benzene, carbon tetrachloride and heptane were employed as weak or nonpolar solvents. Disperse Blue 7 was the disperse dye most strongly adsorbed by the PAC column. It was useful for screening candidate solvents since the mobile phase must be capable of eluting the strongest held solute from the column in a reasonable time. After study of a large number of solvent-nonsolvent pairs, tetrahydrofuran (THF) and cyclohexane (CH) were selected for separating the disperse dyes.

Elution volumes in ml for the disperse dyes on Partisil PAC with a number of tetrahydrofuran-cyclohexane mixtures are shown in Table 4. There is some scatter in the data due to failure of the solvent mixing valve during a few of the runs. The results suggest that a solvent-nonsolvent gradient beginning at a ratio of 25/75 THF/CH and increasing to 100% THF should give good separation of the 7 disperse dyes. This gradient system gave excellent separation of the disperse yellow dyes as can be seen in Figure 1.

Difficulties were encountered in using the gradient system for the disperse red and disperse blue dyes. These dyes are not single compounds and the gradient system separated each of these dyes into a number of components. This problem is illustrated in Figure 2 where at least 6 components are observed for Disperse Blue 7. This separation greatly reduces the signal-to-noise ratio and decreases the sensitivity of the analytical method. Specific solvent ratios were therefore used

Table 4

Elution Volumes (ml) for Disperse Dyes on Partisil PAC

	25/75	30/70	35/65	40/60	50/50	60/40	75/25	80/20	81/19	90/10
DB 120	7.0	8.8	4.9	6.4	3.8		3.5			
DR 60	5.1		4.3		3.6	4.3				
DY 54	6.1		4.9		3.9					
DY 23	10.2		7.8		4.8				3.4	
DB 7								14.9		10.9
DR 55				4.0	9.4	5.2				
DY 3					15.7			3.6	4.9	

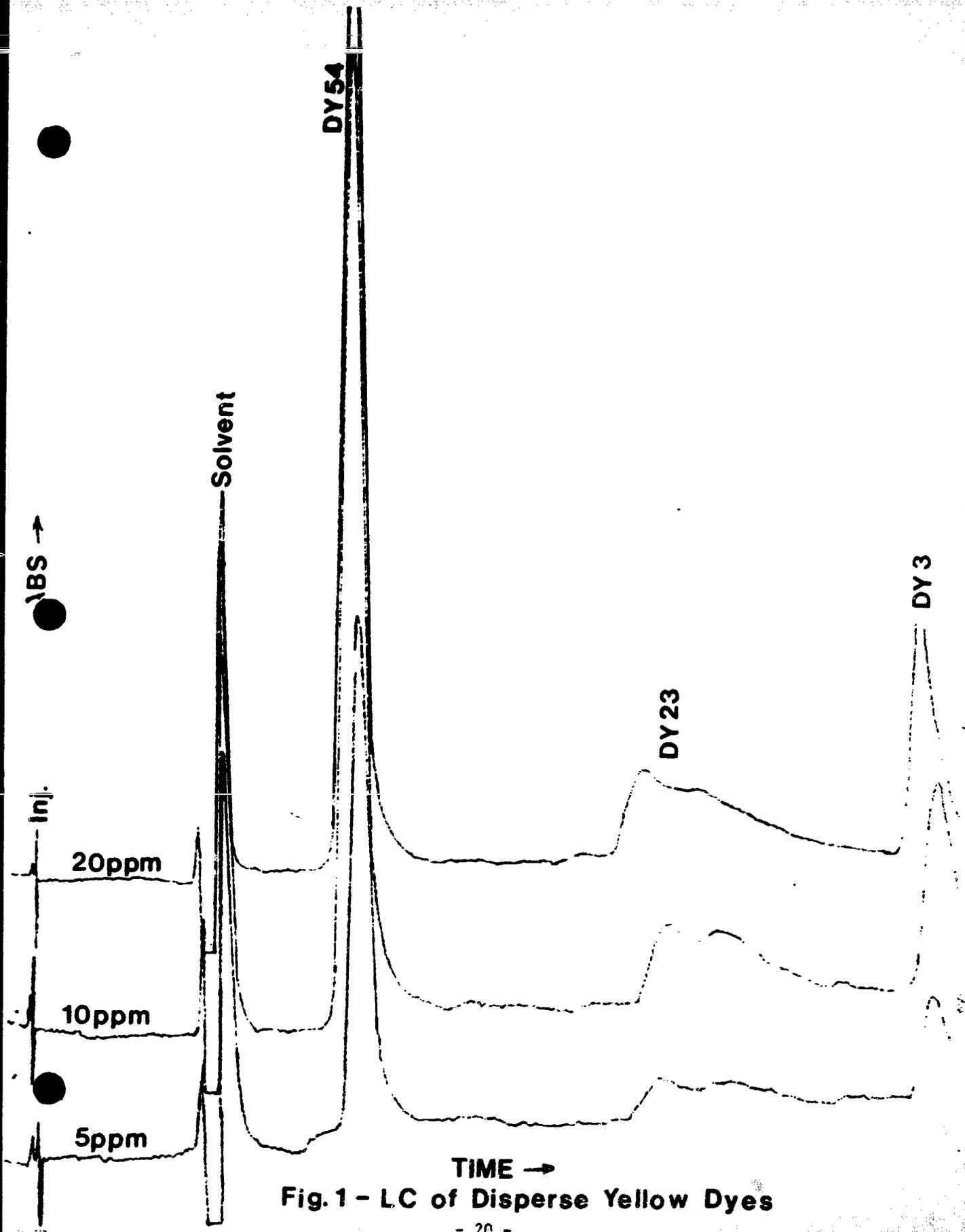


Fig.1 - LC of Disperse Yellow Dyes

TIME →

0 0 2 9

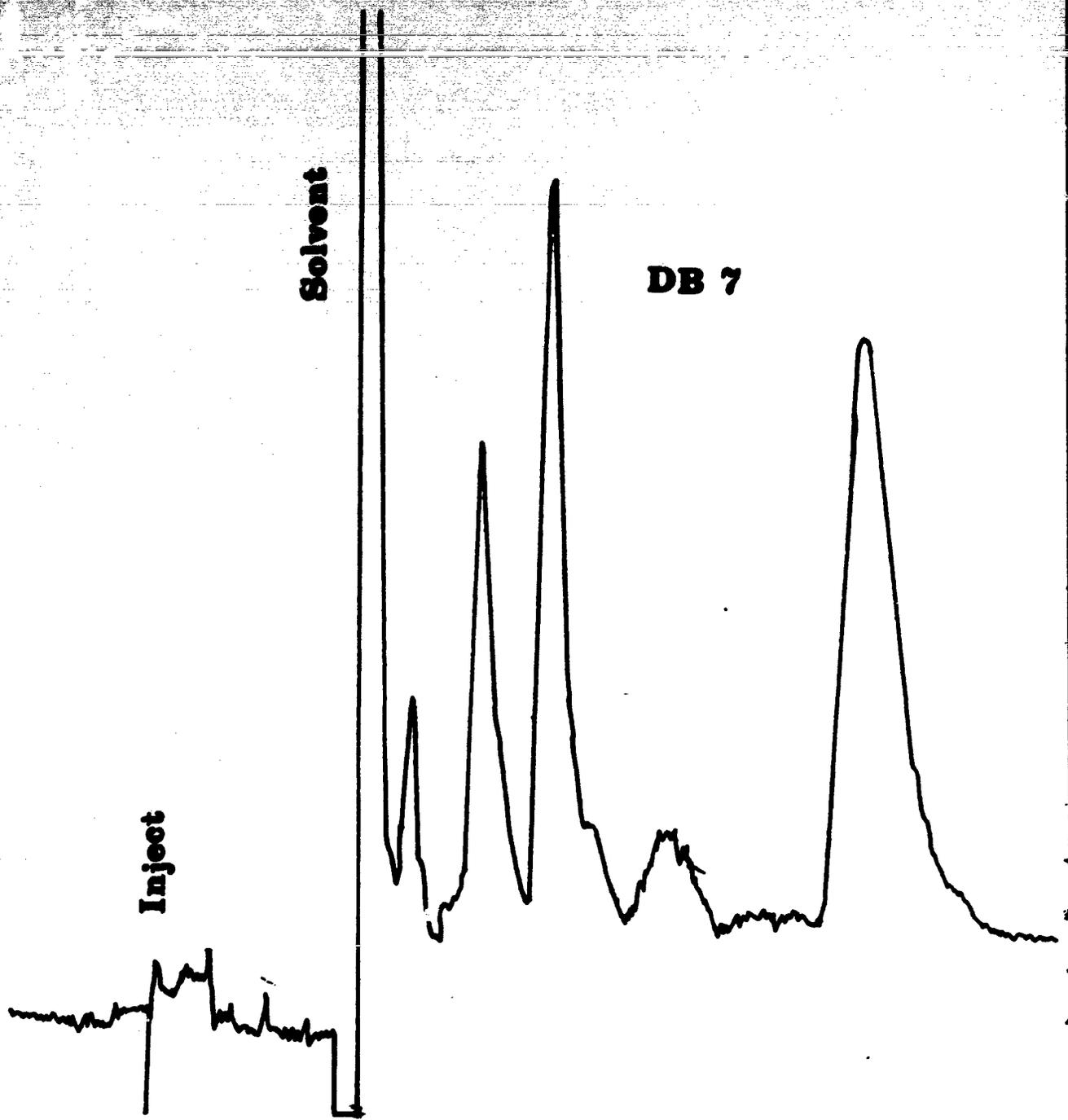


FIG. 2. LIQUID CHROMATOGRAM OF DISPERSE BLUE

to elute the red and blue dyes.

The specific systems used for the disperse dyes are detailed below.

b. Disperse Yellow Dyes

The column is first conditioned by pumping a 25/75 mixture of tetrahydrofuran/cyclohexane through the column for at least 15 minutes. A 20 μ l sample of the dye mixture is injected in the solvent stream and the effluent of the column monitored at 420 nm. The composition of the solvent varied linearly from 25/75 to 100/0 tetrahydrofuran/cyclohexane over a 15 minute period. The solvent flow rate was 1 ml/minute. The resulting chromatograms of a standard 5, 10 and 20 ppm mixture of Disperse Yellow 3, 23, and 54 are shown in Figure 1. The disperse yellows are well separated and excellent linear curves are obtained when the areas under the peaks are plotted versus concentration.

It is interesting to note that Disperse Yellow 23 gives several peaks. This dye is undoubtedly a mixture of several compounds which are partially separated by the chromatograph.

c. Disperse Red Dyes

Two separate runs on the chromatograph were required to analyze for the disperse red dyes at very low concentrations. The probable reason for this was that the red dyes are a mixture of compounds and when long elution times were used the peaks split into many components which reduce the sensitivity.

The following procedure was used with the red dyes. The column was equilibrated for 15 minutes with an 80/20 tetrahydrofuran/cyclohexane solution. Twenty μ l of dye were injected and the effluent monitored at 520 nm. A flow rate of 1 ml/minute was used. Under these conditions Disperse Red 55 elutes with the solvent front and Disperse Red 60 approximately 1 to 2 minutes after the solvent front. The column is then equilibrated with a 35/65 mixture of tetrahydrofuran/cyclohexane and 20 μ l of the dye mixture again injected. Under these conditions Disperse Red 55 elutes just after the solvent front and Disperse Red 60 is retained on the column. Typical chromatograms for 5, 10 and 20 ppm Disperse Red 55 are shown in Figure 3. Very similar results were obtained for Disperse Red 60. It should be noted that the detector was not set at its highest sensitivity in recording Figure 3. At highest sensitivity 1 ppm of the disperse red dyes can be very readily detected and analyzed.

d. Disperse Blue Dyes

A system similar to the one used for the disperse red dyes was used in analysis of disperse blue dyes. The column is equilibrated with 100% tetrahydrofuran at a flow rate of 1 ml/min. A 20 μ l sample of the dye mixture is injected and the effluent monitored at 620 nm. The chromatogram shows a number of peaks indicating that the dye contains several components but two very distinct peaks are observed at about 2 and 4 minutes retention time. Under these conditions Disperse Blue 120 elutes with the solvent front.

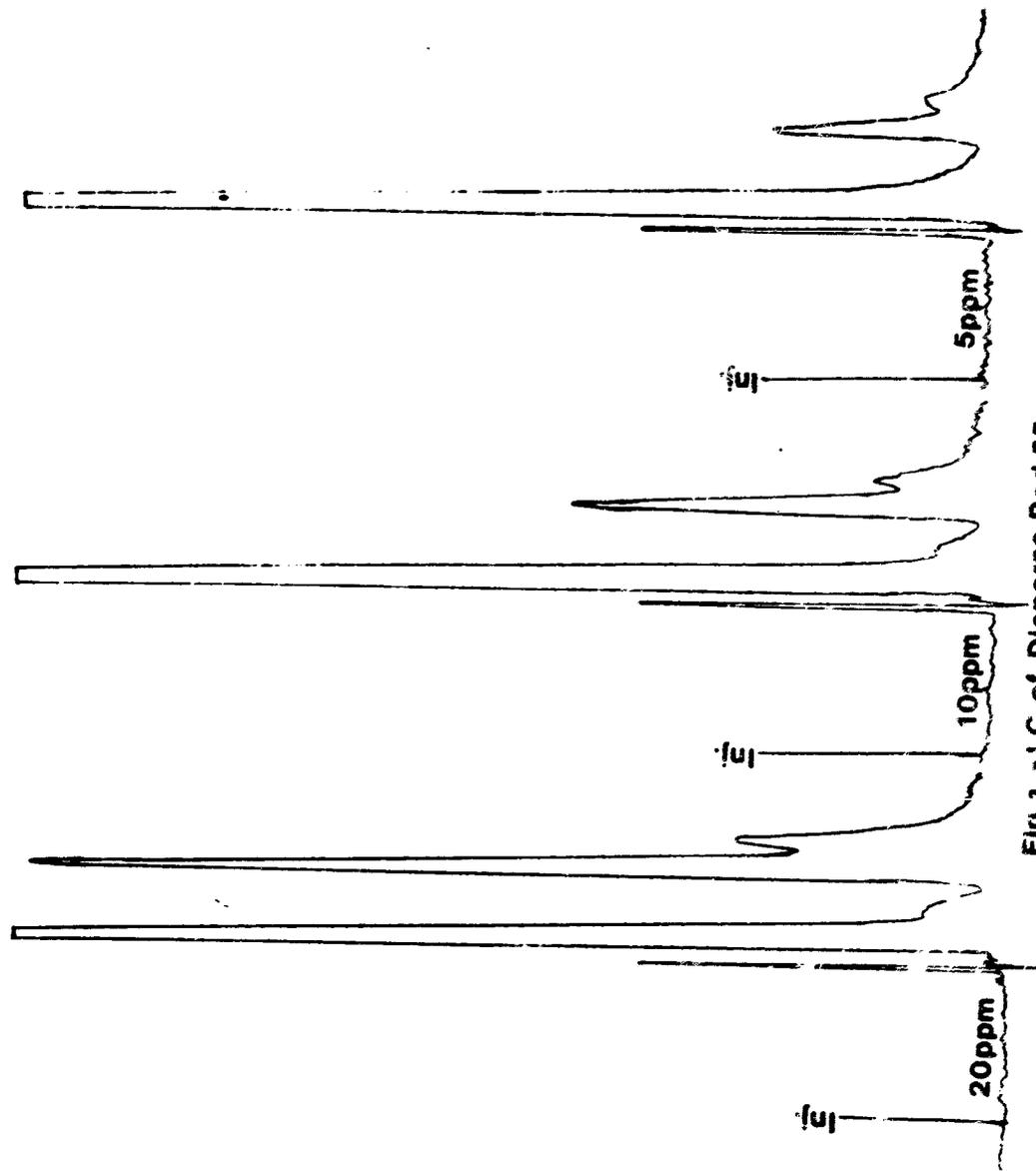


Fig. 3. ^{13}C of Disperse Red 55

Disperse Blue 120 is determined by equilibrating the column with a 45/55 tetrahydrofuran/cyclohexane mixture and eluting with this mixture. Several peaks are observed with the principal peak at about 2 minutes retention time. Under these conditions Disperse Blue 7 is retained by the column thus providing a separation of the two blue dyes. After elution of the Disperse Blue 120 the Disperse Blue 7 is stripped by pumping 100% THF through the column.

3. Analysis for Acid Dyes

Acid dyes usually contain one or more ionizable sulfonate groups as part of the dye structure (see Appendix A). This class of dyes could probably be separated by ion exchange liquid chromatography. However, separations are usually difficult and reproducibility is poor with ion exchange techniques.

After survey of the possible separation procedures, a technique known as paired-ion chromatography (PIC) was selected for investigation [22]. In this technique the dyes are adsorbed from solution on a silica column containing C₁₈ hydrocarbon groups bonded to the silica particles. The dyes are eluted with methanol-water mixtures containing tetrabutylammonium phosphate. This technique has been used to separate food dyes very similar in structure to the dyes of interest in this work [23].

The column used in the acid dye studies was a 25 cm. Spherisorb ODS (5 micron silica particles with a C-18 hydrocarbon bonded to the surface) obtained from Laboratory Data Control. The elution solvents were prepared by dissolving buffered tetrabutylammonium phosphate in methanol

and in water (the buffered tetrabutylammonium phosphate is sold under the name PIC Reagent A by Waters Associates).

Preliminary experiments using the PIC technique were conducted on known mixtures of acid yellow dyes. After several trials it was found that an elution gradient beginning at a 60 to 40 ratio of methanol to water and increasing linearly to 85/15 methanol/water over a 10 minute period gave good separation of the yellow dyes. A flow rate of 1 ml per minute was used and the detector was set at 420 nm. This same gradient was found to separate the acid red dyes and the acid blue dyes. The only change necessary for the red and blue dyes was setting the wavelength of the detector at 520 and 615 nm, respectively. Typical chromatograms for the acid yellow, red and blue dyes are shown in Figures 4, 5, and 6.

4. Quantitation of Acid Dyes

The concentrations of each of the 15 dyes was determined from the areas under the liquid chromatography peaks. The system was calibrated by running standard 5, 10 and 20 ppm solutions of each dye and determining the areas of the peaks. The areas under the peaks were obtained from the recorder integrator. Since the baseline was not always level, the areas determined from the integrator had to be corrected for the background. This was done by running the solvent or solvent program with only a "blank" (i.e., 1% DMF in benzene or methanol, the solvents used for the dyes) injected under identical instrumental conditions as the dyes and subtracting the background correction from the measured dye peak areas. Typical calibration curves are shown in Figures 7 and 8.

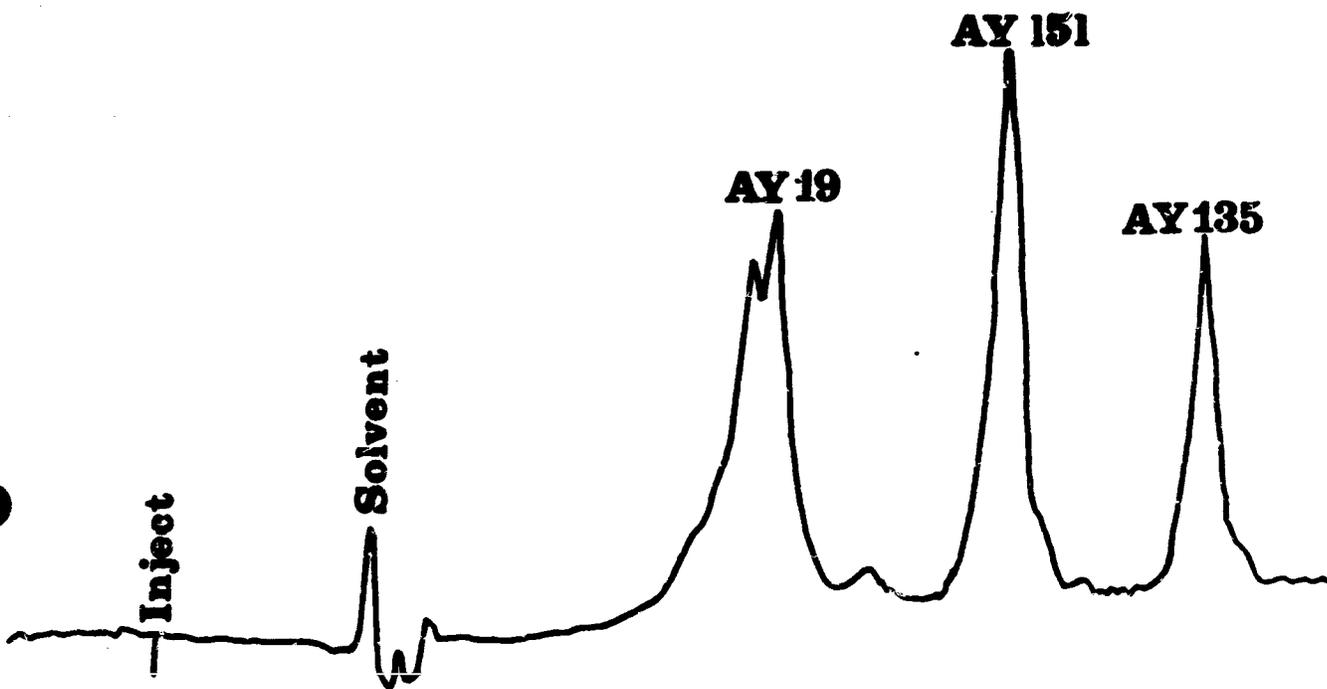


FIG. 4. LIQUID CHROMATOGRAMS OF ACID YELLOW DYES

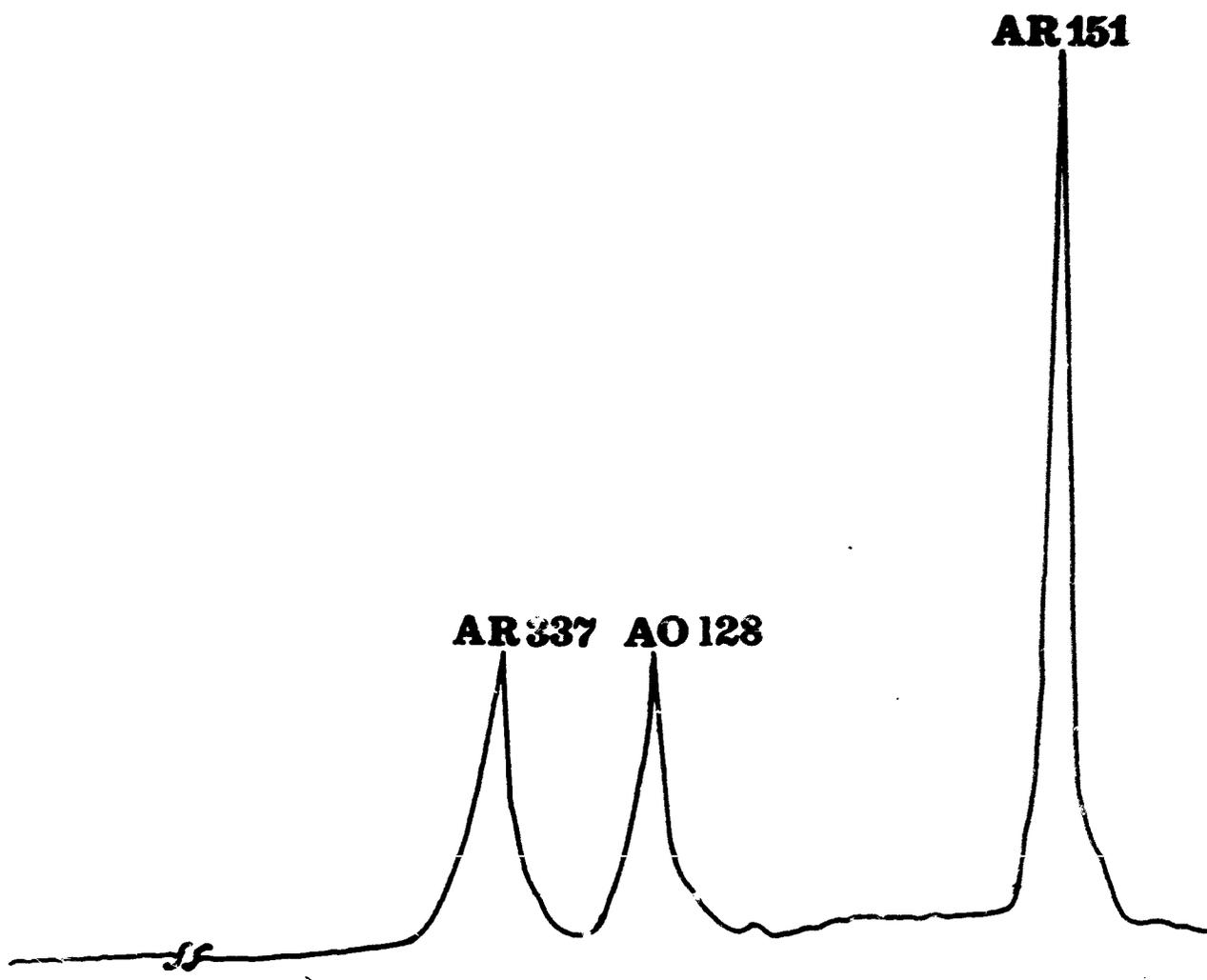


FIG. 5. LIQUID CHROMATOGRAMS OF ACID RED AND ORANGE DYES

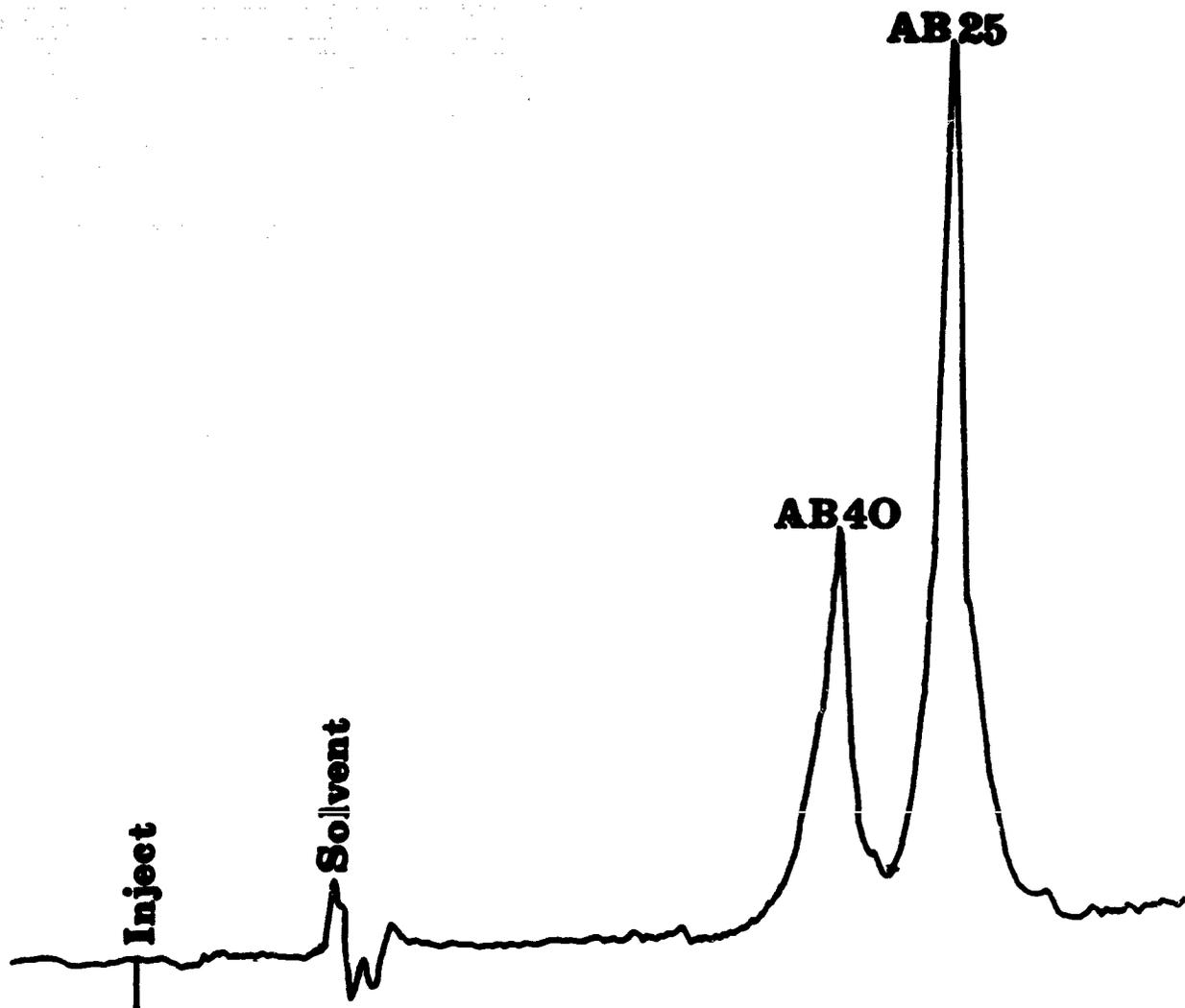


FIG. 6. LIQUID CHROMATOGRAM OF ACID BLUE DYES

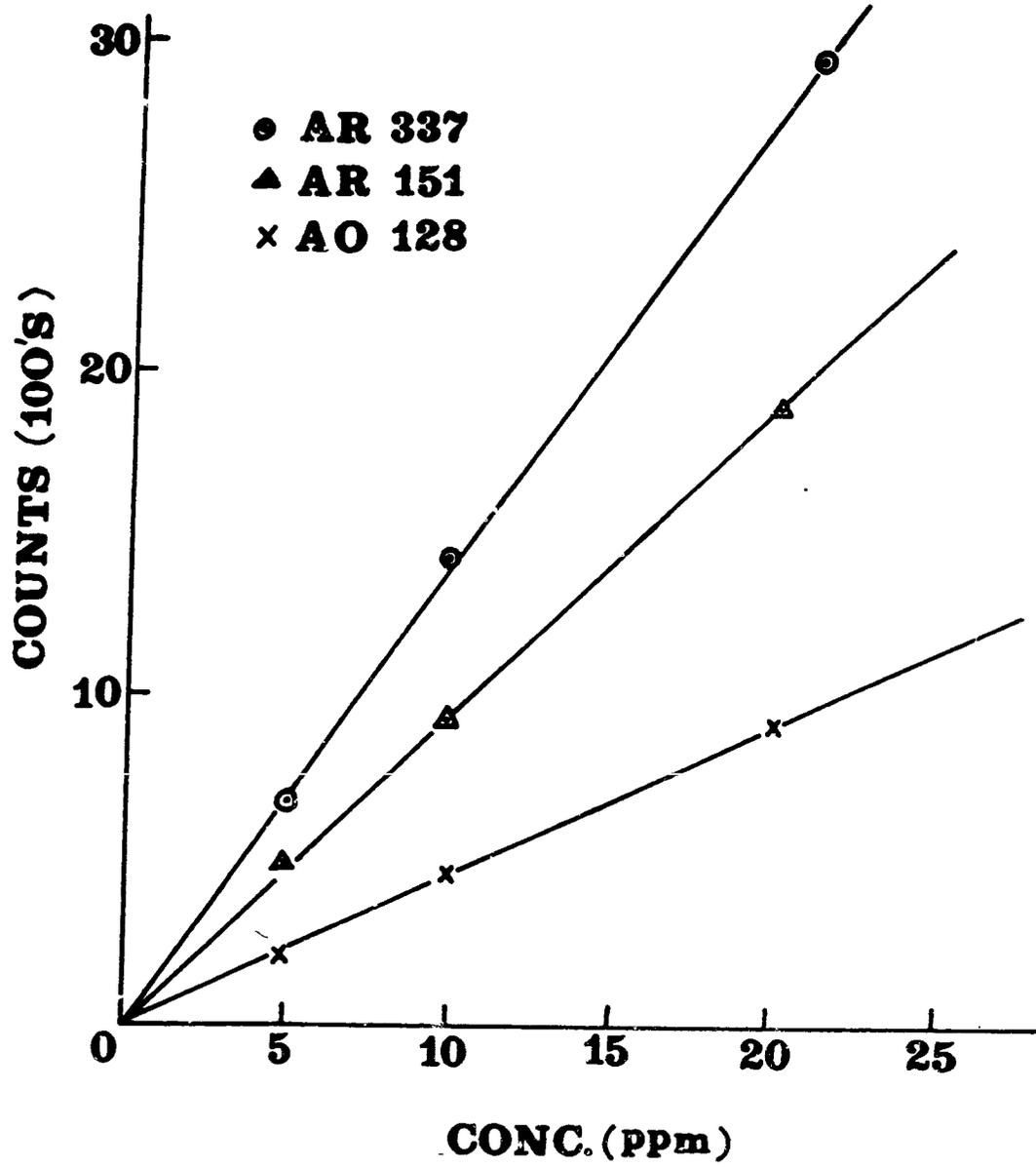


FIG. 7. ANALYTICAL WORKING CURVES FOR ACID RED AND ACID ORANGE DYES.

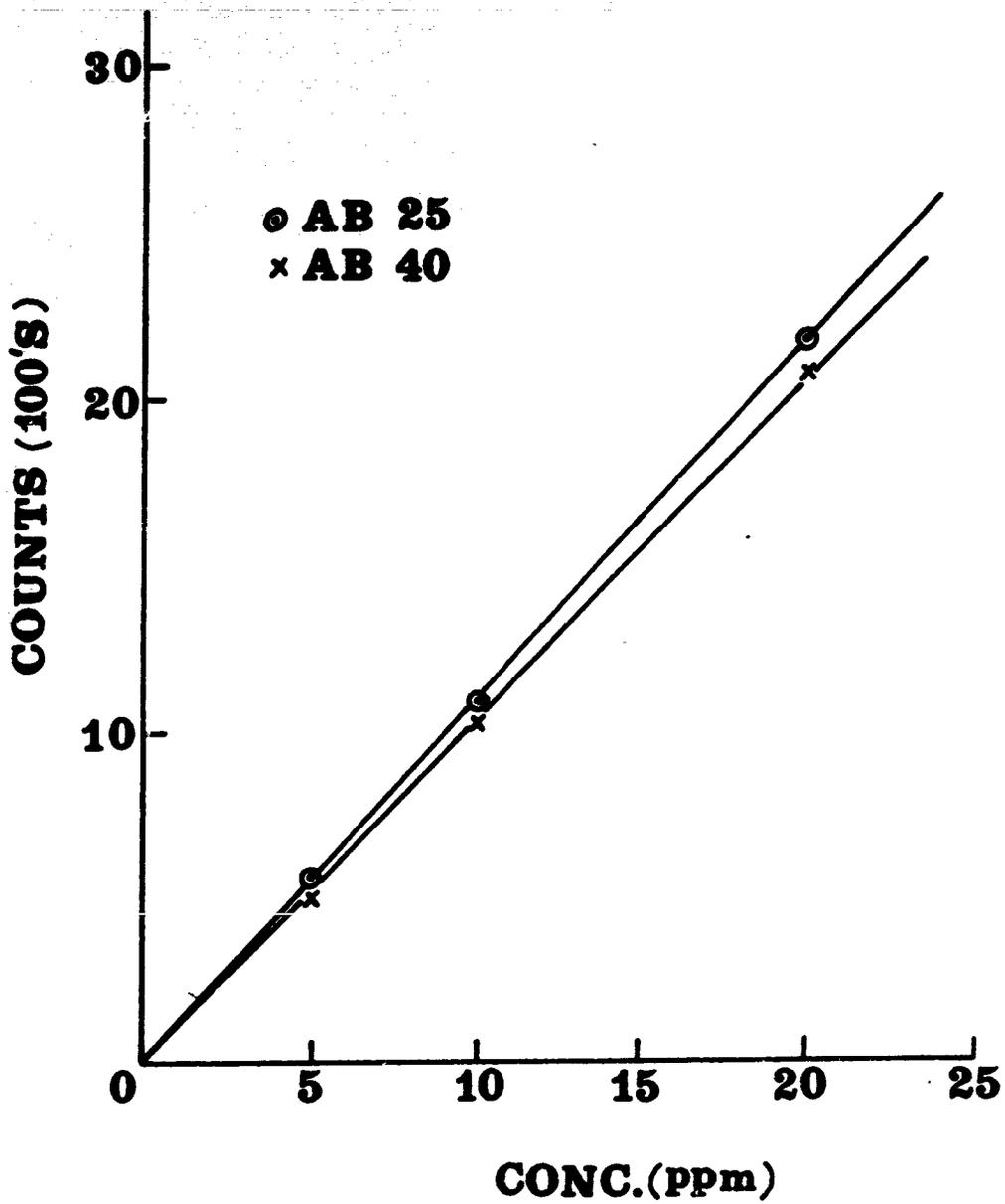


FIGURE 8. ANALYTICAL WORKING CURVES FOR ACID BLUE DYES

0040

One other correction was required for Acid Yellow 151. Acid Orange 128 is the only color in the selected list of dyes that is not a primary color. As a result Acid Orange 128 has strong absorption in both the red and yellow regions of the visible spectrum. This situation creates no problem in analysis of the red dyes since Acid Orange 128 is well separated from Acid Red 337 and Acid Red 151 (see Figure 5). However, in the analysis of the yellow dyes, Acid Orange 128 is not separated from Acid Yellow 151. Two approaches were considered for solution of this problem. First, the selected solvent program for the yellow dyes could have been changed. This approach was not selected as it would make an otherwise very simple analytical scheme for acid dyes more complex. The second approach and the one selected was to correct the AY 151 absorption for the Acid Orange 128. In this scheme the concentration of Acid Orange 128 is determined from the adsorption at 520 nm. From the concentration and the known absorptivity at 420 nm the contribution of Acid Orange 128 at 420 nm can be subtracted to obtain the true absorption due to Acid Yellow 151.

All the other acid dyes have been examined spectrophotometrically to determine if any other similar interferences are present. Acid Orange 128 was the only problem found.

F. Discussion of the Analytical System

1. Sensitivity of the Analytical System

No tests of the absolute sensitivity of the analytical system for disperse and acid dyes have been carried out. However, use of the system for analysis of large numbers of wastewater and laboratory samples suggest that with the exception of Disperse Blue 120 and

Disperse Blue 7 the dyes can be detected by the liquid chromatograph (at highest sensitivity) at approximately 0.1 ppm. With the concentration of dyes in 1800 ml of wastewater in a volume of 10 ml by the resin adsorption procedure (giving a 180-fold increase), the system can detect 13 of the dyes at less than 1 part-per-billion (ppb) in wastewater. Under similar conditions it is estimated that Disperse Blue 7 can be detected at 10 ppb and Disperse Blue 120 at 25 ppb.

2. Performance of the Analytical System

The procedures for determining the concentration of disperse dyes in complex mixtures have been employed on several typical carpet waste samples containing known quantities of disperse dyes. This typical waste sample was prepared by dissolving appropriate quantities of the chemicals given in Table 20 of the report "Chemical Use and Discharge in Carpet Piece Dyeing" [5] in water. The sample contained not only dyes but the auxiliaries, finish components, pH control agents and other organic and inorganic compounds commonly present in dye wastewater. The quantities of each of 6 disperse dyes present in the sample are given in Table 5.

One liter of the synthetic waste sample was concentrated using the column procedure described previously. The concentrate was injected in the liquid chromatograph and the quantities of each of the six dyes found in the known waste sample are given in Table 5 as well as the percent of the known that was found. Results indicate that greater than 70% of most disperse dyes are detected. The results

Table 5

Analysis of Known Mixture

<u>Dye</u>	<u>Conc. in Waste Known (mg/L)</u>	<u>Conc. in Waste Found (mg/L)</u>	<u>% Found</u>
DY 54	5.67	4.05	70
DY 3	5.78	5.31	92
DY 23	8.21	5.44	66
DR 60	3.97	3.44	87
DR 55	1.82	1.27	70
DB 7	1.82	1.18	65

0043

agree closely with the previous studies on dye recovery (Table 3) by the resin adsorption system and suggest that improvements in this system should be investigated.

Part II

Analysis of Coosa River Basin Samples
For Acid and Disperse Dyes

0045

A. Samples

The analytical techniques described in the previous section have been used to analyze a number of samples from the Coosa River basin. The Coosa River and its tributaries carry over 50% of all carpet dyeing wastewater in the United States. A map of the Coosa basin in Georgia is shown in Figure 9. The cities of Dalton, Chatsworth, Calhoun, Cartersville and Rome are all major centers for carpet production. The city of Dalton is the principal center with the Dalton River Bend Waste Treatment plant receiving approximately 25% of all U.S. carpet dyeing wastewater.

Grab samples were collected in the Coosa basin at three different times during 1976-1977. The first set of samples was collected on October 28, 1976, the second set on March 8 and 9, 1977, and the third set on June 7 and 8, 1977. The samples collected March 8 and 9 were collected at times selected to correspond with times at which dyes should have been at peak concentration at the collection site.

The peak period in dyeing of carpet in the Dalton area occurs during the day shift on Mondays through Thursdays. This results in a peak flow at the Dalton waste treatment plant usually between 3 and 5 in the afternoon of those days. Friday is generally a "clean-up" day and most plants are closed on weekends except during peak periods of production. An attempt was made to collect samples in the Coosa basin at times such that the samples would reflect the peak flows at the Dalton waste treatment plant and contain maximum dye concentrations.

Mr. Gary Ellis of the Environmental Protection Division provided flow times in the Coosa basin based on a computer simulation of stream flow. Volumes and flow times based on this model for the week of March 7 are shown in Table 6.

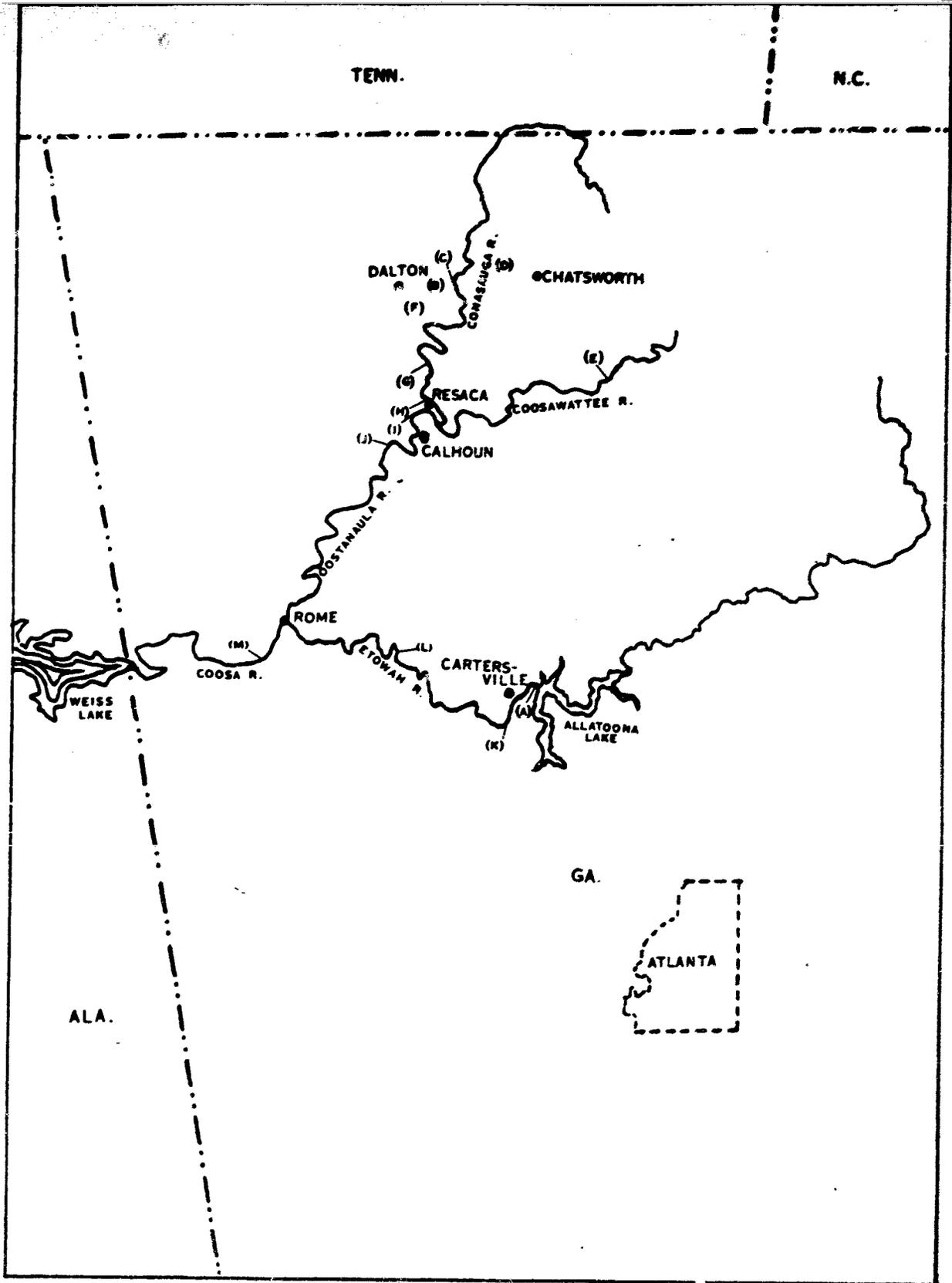


FIGURE 9. -- The Coosa River Basin with location of centers of carpet manufacturing

0047

Table 6

Flow Times in Coosa Basin - March 7, 1978

<u>Site</u>	<u>Flow</u>	<u>Time</u>
Dalton Water Intake	—	0
Tibbs Bridge	52 cfs	13-15½ hours
Dalton Waste Treatment Outfall	—	12-14½ hours
Looper's Bridge	789 cfs	1 hour (from DWTP)
Tilton Bridge	884 cfs	15-18½ hours (from DWTP)
Calhoun Water Intake	2550 cfs	22-26½ hours (from DWTP)
Rome Water Intake	2640 cfs	30-35 hours (from DWTP)

0 0 4 8

During the week selected for sampling the carpet industry in Dalton was operating at 80 - 90% of normal production capacity based on total flow at the Dalton waste treatment plant. Residence time in the Dalton waste treatment plant is approximately 24 hours. The effluent on any given day is therefore representative of the influent received the previous day.

The day and hour of collection at the various sites is shown in Table 7. The table also gives the estimated time that the sample was leaving the Dalton waste treatment plant. The sample collected at the Georgia Highway 40 bridge over the Oostanula River was collected at a time such that the sample was entering the Calhoun Waste Treatment plant Tuesday, March 8 at approximately 2:00 P.M.

All samples were refrigerated at the collection site and were kept at 0°C until the concentration step.

A third sampling trip was conducted on June 7 and 8. This time was selected to take advantage of the low flows experienced in early June. Samples were collected in the Rome area on June 7 and in the Dalton and Calhoun area on June 8. These samples were numbered 129-30-1 to 129-32-5. All samples were refrigerated immediately after collection and remained refrigerated until concentrated except finish water samples. Finish water samples were refrigerated within 8 hours of collection. Three mud samples were collected to determine the quantity of dyestuffs that may be adsorbed on mud in the Coosa basin. These samples required a different extraction procedure as outlined below.

A complete list of all samples collected is given in Table 8. As reflected in the sample collection special emphasis was placed on analysis

Table 7

Sample Collection Times

<u>Site</u>	<u>Time Collected</u>	<u>Time Left</u> <u>Dalton Waste Treatment Plant</u>
129-22-1 Dalton Waste Treatment Plant Influent	Wed., Mar. 9, 4:00 PM	Thur., Mar. 10, 4:00PM
129-22-2 Dalton Waste Treatment Plant Effluent	Wed., Mar. 9, 4:20 PM	Wed., Mar. 9, 4:20PM
129-22-3 Looper's Bridge	Wed., Mar. 9, 5:00 PM	Wed., Mar. 9, 4:00PM
129-22-4 Tilton Bridge	Thurs., Mar. 10, 9:00 AM	Wed., Mar. 9, 4:00PM
129-23-5 Calhoun Raw Water	Thurs., Mar. 10, 10:00 AM	Tues., Mar. 8, 4:00PM
129-23-6 Calhoun Finish Water	Thurs., Mar. 10, 10:30 AM	Tues., Mar. 8, 4:00PM
129-23-7 Calhoun Filter Backwash Plant Influent	Thurs., Mar. 10, 11:00 AM	Tues., Mar. 8, 4:00PM
129-23-8 Calhoun Waste Treatment Plant Influent	Thurs., Mar. 10, 12:30 PM	— —
129-23-9 Calhoun Waste Treatment Plant Effluent	Thurs., Mar. 10, 12:50 PM	— —
129-23-10 Georgia Highway 40 Bridge (Oostanaula)	Thurs., Mar. 10, 2:00 PM	— —

Table 8

Coosa Basin Samples Collected

<u>Sample</u>	<u>Collection Site</u>	<u>Time of Collection</u>	
129-10-2	Dalton Raw Water Intake	10/28/76	11:45 A.M.
129-11-5	Dalton Waste Treatment Plant Influent	10/28/76	3:15 P.M.
129-11-8	Dalton Waste Treatment Plant Effluent	10/28/76	4:15 P.M.
129-12-9	Looper's Bridge	10/28/76	5:15 P.M.
129-22-1	Dalton Waste Treatment Plant Influent	3/9/77	4:00 P.M.
129-22-2	Dalton Waste Treatment Plant Effluent	3/9/77	4:20 P.M.
129-22-3	Looper's Bridge	3/9/77	4:45 P.M.
129-23-4	Tilton Bridge	3/10/77	9:00 A.M.
129-23-5	Calhoun Raw Water Intake	3/10/77	10:00 A.M.
129-23-6	Calhoun Finish Water	3/10/77	10:30 A.M.
129-23-7	Calhoun Filter Backwash	3/10/77	11:00 A.M.
129-23-8	Calhoun Waste Treatment Plant Influent	3/10/77	12:30 P.M.
129-23-9	Calhoun Waste Treatment Plant Effluent	3/10/77	12:50 P.M.
129-23-10	Oostananla Bridge at Ga. Highway 140	3/10/77	1:45 P.M.
129-30-1	Rome Raw Water Intake	6/7/77	3:20 A.M.
129-30-2	Rome Finish Water	6/7/77	9:35 A.M.
129-30-3	Rome Waste Treatment Plant Influent	6/7/77	3:35 P.M.
129-30-4	Rome Waste Treatment Plant Effluent	6/7/77	4:00 P.M.
129-30-5	Looper's Bridge	6/7/77	5:45 P.M.

Table 8 (cont'd.)

<u>Sample</u>	<u>Collection Site</u>	<u>Time of Collection</u>	
129-31-1	Looper's Bridge Mud Sample	6/8/77	6:00 PM
129-31-2	Tilton Bridge	6/8/77	10:30 A.M.
129-31-3	Tilton Bridge Mud Sample	6/8/77	10:55 A.M.
129-32-1	Calhoun Water Intake	6/8/77	11:30 A.M.
129-32-2	Calhoun Water Intake Mud Sample	6/8/77	11:30 A.M.
129-32-3	Calhoun Finish Water	6/8/77	11:45 A.M.
129-32-4	Calhoun Waste Treatment Plant Influent	6/8/77	1:15 P.M.
129-32-5	Calhoun Waste Treatment Plant Effluent	6/8/77	1:30 P.M.

of city water supplies in the Coosa basin.

B. Analysis of Collected Water and Wastewater Samples

The collected stream, finish water, and filter backwash water samples were concentrated using the XAD-2 resin system. These samples were concentrated 180-fold (1800 ml \rightarrow 10 ml). The influent and effluent waste treatment plant samples were generally concentrated only 36-fold (1800 ml \rightarrow 50 ml) due to the higher dye concentrations in these samples.

Twenty microliter samples of the extracts were injected into the liquid chromatograph and analyzed by the procedure previously described. Results of the analysis for disperse dyes are given in Table 9 and for acid dyes in Table 10. It should be noted that, due to a loss of part of the concentrate, data on disperse dyes were not obtained for samples 129-11-8 and 129-12-9.

C. Analysis of Mud Samples

During the June 7 and 8 sampling trip, mud samples were collected at the Looper's Bend Bridge, at the Tilton Bridge and near the intake of the Calhoun water treatment plant. The dry mud samples were concentrated by placing 100 gram samples in a Soxhlet extractor and extracting with benzene followed by methanol and then by the pyridine, ammonium hydroxide and tetrahydrofuran mixture for 24 hours. The same extracting solvent was used for subsequent 100 gram samples until a total of 1000 grams of mud had been extracted. The extracts were rotavapped to dryness and taken up in either 50 or 25 ml of solvents for liquid chromatographic analysis. The concentration factor for samples 129-31-3 and 129-32-1 was 40 and for sample 129-31-1 the factor was 20.

Table 9

Concentration of Disperse Dyes (parts-per-billion) in
Samples Taken from the Coosa River Basin

<u>Sample</u>	<u>DY 3</u>	<u>DY 23</u>	<u>DY 54</u>	<u>DR 55</u>	<u>DR 60</u>	<u>DB 7</u>	<u>DB 120</u>
129-10-2	-	-	-	-	-	-	-
129-11-5	32	458	27	187	78	97	198
129-22-1	77	36	7	67	16	62	<25
129-22-2	+	17	9	24	14	35	<25
129-22-3	<1	3	+	-	+	-	-
129-23-4	<1	3	+	+	-	-	-
129-23-5	-	-	-	-	-	-	-
129-23-6	<1	-	-	-	-	-	-
129-23-7	<1	-	-	-	+	-	-
129-23-8	436	257	6	38	6	85	38
129-23-9	17	10	2	26	6	152	37
129-23-10	1	-	-	-	+	-	-
129-30-1	1	+	-	-	-	-	-
129-30-2	-	-	-	-	-	-	-
129-30-3	222	93	13	4	52	19	57
129-30-4	256	120	32	11	76	26	114
129-30-5	3	2	5	3	-	22	<25
129-31-2	<1	-	-	-	-	-	-
129-32-2	-	-	-	-	-	-	-
129-32-3	-	-	-	-	-	-	-
129-32-4	209	77	3	86	21	239	56
129-32-5	+	36	<1	20	-	139	56

- dye not detected

+ dye detected but below minimum concentration for
quantitation

0054

Table 10

Acid Dye Concentration (in ppb) in
Coosa Basin Samples

Sample	AY-19	AY-151	AY-135	AB-40	AB-25	AR-337	AO-128	AR-151
129-10-2	+	-	-	-	-	-	-	-
129-11-5	1440	+	-	58	53	101	41	7
129-11-8	378	+	-	-	11	190	17	8
129-12-9	156	+	-	-	-	26	-	2
129-22-1	1200	1110.	-	114	145	-	-	62
129-22-2	617	104	-	352	245	1020	+	77
129-22-3	+	8	-	21	16	42	-	8
129-23-4	62	-	-	29	15	42	-	3
129-23-5	38	-	-	-	-	8	-	1
129-23-6	-	-	-	-	-	-	-	-
129-23-7	-	-	-	-	-	-	-	-
129-23-8	127	57	+	253	54	56	137	7
129-23-9	212	132	+	403	74	28	269	76
129-23-10	-	-	-	-	-	+	-	+
129-30-1	10	-	-	-	-	-	-	-
129-30-2	-	-	-	-	-	-	-	-
129-30-3	23	134	-	+	18	-	49	5
129-30-4	301	3750	-	22	502	78	1120	48
129-30-5	9	+	-	7	42	89	15	1
129-31-2	172	+	-	+	14	19	-	-
129-32-2	32	-	+	-	-	5	-	-
129-32-3	-	4	-	-	+	-	-	-
129-32-4	99	119	130	87	32	13	-	-
129-32-5	172	-	-	197	27	-	+	-

- dye not detected

+ dye detected but below minimum concentration for quantitation

0.055

Results of the analysis of the mud samples for disperse dyes is given in Table 11. Results of the analysis for acid dyes is given in Table 12.

D. Discussion of Results

The immediate conclusion which the results suggest is that acid dyes are present in higher concentration than disperse dyes in the Coosa River basin. There are probably 2 factors accounting for this difference. First, the carpet industry is tending more toward use of acid dyes for dyeing nylon carpet. This is due in part to the increasing use of continuous dye ranges which require acid dyes. Also, acid dyes are being used with increasing frequency in beck dyeing to meet more stringent light and ozone stability requirements. Second, later studies (see Part III) suggest that disperse dyes are more readily removed by biological waste treatment systems than acid dyes. Similarly, since disperse dyes are not in solution but are dispersed as small particles in water, they are probably more readily removed by stream sediments than acid dyes. This is suggested by comparison of the results of the analysis for acid and disperse dyes on the mud samples (Tables 11 and 12).

The influents and effluents of the Dalton, Calhoun and Rome waste treatment plants show appreciable concentrations of all 15 dyes with the exception of Acid Yellow 135. This dye is quite easily detected in the analytical system so its absence suggests that it is not being used as extensively in carpet dyeing as was the case previously. It is interesting to note that

Table 11

Analyses of Mud Samples for Disperse Dyes
(parts-per-billion based on dry mmo weight)

<u>Sample No.</u>	<u>Site</u>	<u>DY 3</u>	<u>DY 23</u>	<u>DY 54</u>	<u>DR 55</u>	<u>DR 60</u>	<u>DB 7</u>	<u>DB 120</u>
129-31-1	Looper's Bridge	420	2970	1600	-	3400	1405	3000
129-31-3	Tilton Bridge	455	1350	970	+	1050	625	3250
129-32-1	Calhoun Water Intake	140	114	31	119	19	-	62

- dye not detected

+ dye detected but below minimum concentration for quantitation

0 0 5 7

Table 12

Analysis of Mud Samples for Acid Dyes
(parts-per-billion)

<u>Sample No.</u>	<u>Site</u>	<u>AY-19</u>	<u>AY-151</u>	<u>AY-135</u>	<u>AB-40</u>	<u>AB-25</u>	<u>AR-337</u>	<u>AD-128</u>	<u>AP-151</u>
129-31-1	Looper's Bridge	2080	515	+	225	550	205	790	27
129-31-3	Tilton Bridge	1168	1105	+	113	315	173	615	35
129-32-1	Calhoun Water Intake	110	165	-	-	+	-	-	15

- dye not detected

+ dye detected not below minimum concentration for quantitation

0 0 5 8

disperse dyes are in relatively higher concentration at Rome and Calhoun and acid dyes in higher concentrations at Dalton. This probably reflects differences in dyeing processes (i.e., more continuous dyeing at Dalton) in the three cities.

In several cases the waste treatment plant effluents show higher dye concentrations than the influents. Since all samples were "grab" samples this is undoubtedly due to daily and time of day variation in concentration of the various dyes. Wide fluctuation in acid dye concentrations can result from discharge of unused dye paste at the end of continuous runs.

Downstream below the outfall of the Dalton waste treatment plant, river samples collected at Looper's Bridge and Tilton Bridge show low concentrations of a few acid and disperse dyes. No disperse dyes were present in the raw water intake at the Calhoun water treatment plant. Small quantities of Acid Yellow 19 and traces of Acid Red 337 and Acid Red 151 were present in the Calhoun raw water. Calhoun finish water was free of dye except for one sample which contained 4 ppb of Acid Red 151. The Rome raw water contained 10 ppb of Acid Yellow 19 and a trace of Disperse Yellow 3 but no dyes were detected in the Rome finish water.

Mud samples collected at Looper's Bridge, Tilton Bridge and at the Calhoun raw water intake all show appreciable quantities of both acid and disperse dyes.

The results suggest that the developed analytical system can be readily applied to dye analysis for both waste treatment plant and stream samples. Analysis of composite samples will probably be necessary to obtain the best data on dye concentrations.

Part III

Removal of Dyes from Carpet Dyeing
Wastewater by Biological and Advanced
Treatment Systems

0 0 6 0

A. Samples

The system of analyses for acid and disperse dyes has been applied to several carpet dyeing wastewater samples that have been subjected to various waste treatment procedures.

Initial studies were carried out on synthetic carpet waste samples treated in a bench scale model of an activated sludge system [24]. The synthetic waste sample was prepared by mixing appropriate quantities of the dyes and chemicals listed in Table 20 of Reference 5. The synthetic carpet dyeing wastewater was fed continuously to a Horizon Bio-Oxidation System [25]. The system was seeded with a sample of acclimated sludge from the Dalton waste treatment plant. After allowing the bio-oxidation system to achieve equilibrium, samples of effluent were collected over a 10 day period and analyzed for 6 disperse dyes. The influent and 10 samples of the effluent were analyzed. The results are shown in Table 13 (the data for Disperse Blue 7 are for the major component which elutes first in the liquid chromatogram). The results suggest that biological oxidation can remove better than 60% of most disperse dyes from dyeing wastewater. The mechanism of removal is probably adsorption on the sludge rather than true biological oxidation.

Dye analysis was also used to evaluate, in part, the effectiveness of various advanced treatment systems in removal of disperse and acid dyes from carpet dyeing wastewater. The treatment systems were set-up at the Dalton River Bend Waste Treatment Plant as part of a research project conducted under the direction of Wiedeman and Singleton Consulting Engineers.

Table 3

Removal of Disperse Dye by Biological Oxidation
of Carpet Dye Wastewater

Sample	DY 54	DY 3	DY 23	DR 55	DR 60	DB 7
	Mg/L Removal					
Influent	4.05	5.31	5.44	1.27	3.44	1.16
Effluent 1	0.98	0.95	1.13	0.23	2.46	0.29
Effluent 2	1.45	0.53	1.85	0.32	2.77	0.53
Effluent 3	0.74	0.37	2.34	0.29	1.48	0.60
Effluent 4	0.73	0.37	1.79	0.24	1.33	0.66
Effluent 5	0.68	0.21	2.28	0.11	0.88	0.53
Effluent 6	0.96	0.16	1.90	0.22	0.48	0.32
Effluent 7	0.73	0.22	1.47	0.19	1.42	0.45
Effluent 8	1.01	0.32	1.74	0.29	1.22	0.41
Effluent 9	0.88	0.16	1.82	0.24	1.06	0.28
Effluent 10	1.03	0.32	2.12	0.62	0.84	0.16

The samples analyzed as part of this project were collected as indicated in Figure 10. The samples are identified in Table 14. The Reactor Effluent samples were collected at the clarifier effluent of the Dalton waste treatment plant (extended aeration activated sludge system). This effluent was then subjected to dual media filtration (24518 and 24705) followed by ozone treatment (24519 and 24706). The reactor effluent was also subjected to flocculation followed by sedimentation (19189 and 19237) and to dual media filtration followed by carbon adsorption (19188 and 19240). All samples were 48 hour composites collected in 1 gallon glass containers. They were refrigerated at 0°C until subjected to the resin concentration procedure.

B. Results of Analysis for Acid and Disperse Dyes

The wastewater samples from the Dalton waste treatment plant and samples of effluents from the various advanced treatment processes were concentrated by macroreticular resin adsorption and the concentrates (dye in 1800 ml wastewater concentrated in 10 ml) separated and quantitated by liquid chromatography as detailed in Part I. Results of the analysis for disperse dyes are given in Table 15. Results of acid dye analysis are shown in Table 16.

C. Discussion of Results

As indicated in Table 15, the concentration of disperse dyes in the effluent of the Dalton waste treatment plant are very low. This probably again reflects the effectiveness of an activated sludge treatment system for removal of disperse dyes. The levels of disperse dyes in these reactor

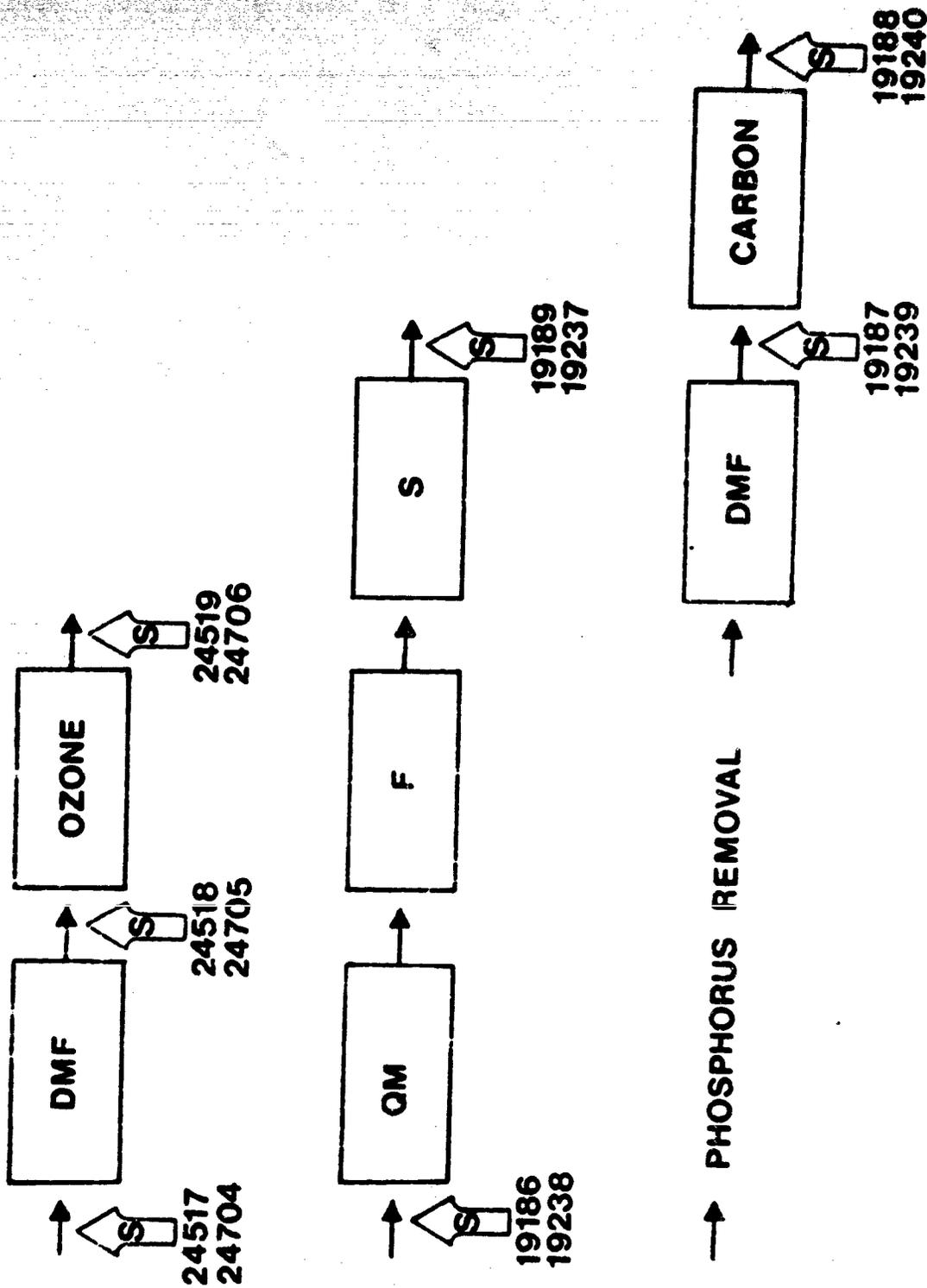


FIGURE 10. SAMPLES COLLECTED FOR EVALUATION OF ADVANCED WASTE TREATMENT SYSTEMS

Table 14

Samples for Dye Analysis

<u>Sample</u>	<u>Location</u>	<u>Date</u>
24517	Reactor Effluent	July 5-7
24518	Dual Media Filter Effluent	July 5-7
24519	Ozone Treatment Effluent	July 5-7
24704	Reactor Effluent	July 12-14
24705	Dual Media Filter Effluent	July 12-14
24706	Ozone Treatment Effluent	July 12-14
19186	Reactor Effluent	May 18-20
19189	Mixed Liquor Before P Removal	May 18-20
19187	Dual Media Filter Effluent	May 18-20
19188	Activated Carbon Effluent	May 18-20
19238	Reactor Effluent	May 23-25
19237	Mixed Liquor Before P Removal	May 23-25
19239	Dual Media Filter Effluent	May 23-25
19240	Activated Carbon Effluent	May 23-25

0065

Table 13

Disperse Dyes in Samples Collected in Dalton
Waste Treatment Plant Study

(ppb)

<u>Sample</u>	<u>DY 3</u>	<u>DY 23</u>	<u>DY 54</u>	<u>DR 55</u>	<u>DR 60</u>	<u>DB 7</u>	<u>DB 120</u>
19186	+	- *	1.8	3.0	5.5	+	-
19187	+	- *	1.3	3.0	2.3	+	-
19188	+	+	+	+	+	-	-
19189	+	- *	+	+	+	-	-
19237	+	+	+	+	+	+	-
19238	+	2.2	+	-	+	+	-
19239	+	1.6	+	-	+	+	-
19240	1.9	2.8	+	-	+	-	-
24517	+	+	2.1	+	+	-	-
24518	+	+	1.1	+	+	-	-
24519	+	1.2	+	-	1.7	-	-
24704	+	+	1.4	1.6	+	+	-
24705	+	- *	1.1	2.3	+	+	-
24706	+	1.8	-	2.0	+	-	-

+ dye detected but concentration less than 1 ppb

- dye not detected

* dye may be present in very low concentration but interference by an impurity prevents quantitation.

Table 16

Acid Dyes in Samples Collected in Dalton
Waste Treatment Plant Study
(ppb)

<u>Sample</u>	<u>AY19</u>	<u>AY151</u>	<u>AY135</u>	<u>A0128</u>	<u>AR337</u>	<u>AR151</u>	<u>AB40</u>	<u>AB25</u>
24517	+	-	-	+	263	+	33	116
24518	-	190	-	+	268	+	47	107
24519	285	-	-	+	104	-	15	46
24704	-	-	-	56	541	4	174	209
24705	-	-	-	28	443	-	178	186
24706	+	-	-	-	-	-	-	-
19186	302	19	-	13	49	4	32	88
19189	-	63	8	+	21	5	8	17
19187	+	9	-	+	14	+	54	214
19188	+	-	-	-	+	-	+	7
19238	-	-	-	-	499	4	44	182
19237	+	32	-	14	19	11	18	33
19239	+	+	-	+	427	2	54	144
19240	-	-	-	-	+	2	14	11

+ dye detected but concentration less than 1 ppb

- dye not detected

effluent samples are lower than those observed previously for Dalton waste treatment plant effluents (see Part II). This difference is probably due to the fact that samples analyzed in Part II were grab samples taken at the time of peak carpet dyeing waste flow and should reflect maximum dye concentrations. The samples collected for this study were composites taken over a 48 hour period and more nearly reflect average concentrations*.

Although the disperse dye concentrations are all quite low, analysis of the advanced treatment system effluents suggest that with the exception of flocculation and sedimentation these treatment techniques are not particularly effective in removing disperse dyes from wastewater.

The results of the analysis for acid dyes (Table 16) confirm previous results (Part II) that acid dye concentrations are higher in the Dalton waste treatment plant effluents than disperse dye concentrations. The acid dye concentrations show wide fluctuations among the various waste treatment plant effluent samples. These fluctuations may result from discharge of concentrated dye solutions used in continuous dyeing of carpet.

Analysis of the advanced treatment systems effluents suggest that dual media filtration is virtually useless in removing acid dyes (compare Samples 24517 with 24518 and Samples 24704 with 24705). Ozone treatment is effective as can be seen from comparison of Samples 24518 and 24519 and Samples 24705 and 24706. Carbon adsorption is also very effective for removal of acid dyes (compare 19187 with 19188 and 19239 with 19240).

One of the more interesting conclusions from this work is that either ozone or carbon adsorption treatment following biological oxidation should provide a good system for removal of dyes from carpet dyeing wastewater.

* It was learned later that these samples had been chemically coagulated and clarified prior to submission for analysis [29]

Biological treatment should effectively remove disperse dyes and the ozone or carbon adsorption remove acid dyes. Carbon adsorption in conjunction with biological treatment appears to be particularly effective as indicated by the very low dye concentrations in samples 19188 and 19240.

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PART IV

**Improvement and Extention of
the Dye Analysis System**

A. Introduction

As noted in Part I, the recovery of dyes from the macroreticular resin column is one aspect of the analytical system which could be improved. Although recoveries of all dyes were greater than 70%, it was felt that this could be improved by modifications in the recovery procedure. This aspect of the dye analysis system was investigated for more efficient dye recovery.

Extension of the dye analysis system to a second major class of dyes, direct dyes, has been investigated also. Direct dyes are used extensively in dyeing of cotton textiles and are the major dyes used in coloration of paper products. The similarity of structure between acid dyes and direct dyes suggested that the analytical system might also be applicable to this important dye class.

B. Improved Dye Recovery System

Spectrophotometric analysis of the column effluents from the XAD-2 resin adsorption of dye from water-DMF solvent mixtures showed that acid and disperse dyes are quantitatively removed by the resin. However, recovery of the dye from the resin column by backwashing with selected solvents did leave some dye remaining on the column. This was evident from the slight color change in the resin that could be observed after backwashing. Alternative methods for removal of the tightly bound dye from the resin were, therefore, investigated.

In previous work the resin column was inverted and backwashed with selected solvents to remove the disperse and acid dyes.

An improved technique for removal of a mixture of dyes adsorbed on macroporous resins based on a Soxhlet extraction process was attempted. The dried resin collected in the extraction thimble was placed in a Soxhlet unit and extracted using selected solvents. The resin was first extracted for four hours using benzene to remove disperse dyes. The resin was removed, dried, and returned to the Soxhlet unit again. The acid dyes were removed by extraction with methanol and then with pyridine-THF-1% NH_4OH (40:40:20). In this separation system, the recovery efficiency was excellent for disperse dyes but was poor for acid dyes. Other techniques were studied, therefore, for removal of acid dyes. Extractions using pure pyridine and immersion of resins in solvent mixtures of THF-1% NH_4OH and MeOH-1% NH_4OH prior to extraction were tried. None of these procedures gave good removal of acid dyes. Finally, an improved technique for the analysis of acid dyes was developed. After removal of the disperse dyes with benzene, a mixed solvent containing 90-ml of pyridine - 2% NH_4OH (50:50) was added to the extraction thimble. This system was allowed to stand for one hour. The acid dyes were then removed from the resin by extraction with boiling pyridine. The dyes removed by benzene (disperse dyes) and pyridine (acid dyes) were further concentrated by evaporation of solvent in a rotary evaporator. The solid dyes obtained from vaporization were prepared for analysis by taking them up in a DMF 1% - benzene 99% solution for disperse dyes, and in a DMF 1% - methanol 99% solution for acid dyes. Twenty five mls of solution were prepared for standard samples giving a concentration factor of 40. The dyes

were separated and quantitated using the high pressure liquid chromatograph described previously.

To determine recovery efficiency 1 liter (water 900-ml - DMF 100 ml) of 1 ppm concentrations of each of fifteen dyes was analyzed as described above. The results are shown in Table 17. These data show that the recoveries are 100% for Disperse Yellow 3, Disperse Red 55, Disperse Red 60, Acid Red 337, and Acid Orange 128; above 95% for Disperse Blue 7, Acid Yellow 135, Acid Yellow 151, Acid Red 151, and Acid Blue 25; between 90% to 95% for Disperse Yellow 23, Disperse Yellow 54, and Acid Blue 40; and about 82% for Disperse Blue 120 and Acid Yellow 19. The recoveries are considerably better than those achieved in previous work as can be seen by comparing Table 17 and Table 18. Further details on this work are available in reference 26.

C. Extension of the Analytical System to Direct Dyes

Direct dyes are used extensively in dyeing both textiles and paper products. Discussions with manufacturers of direct dyes suggested that the following direct dyes are used in very large volume:

Direct Yellow 105

Direct Yellow 106

Direct Red 80

Direct Red 81

Direct Blue 98

Direct Blue 218

These six dyes were selected for initial studies to develop an analytical system for direct dyes. Structures of four of these dyes have been published

Table 17. Recovery for Disperse and Acid Dyes Analyzed by the Improved Analytical System*

Disperse Dyes	Recovery (%)	Acid Dyes	Recovery (%)
Disperse Yellow 3	100	Acid Yellow 19	82
Disperse Yellow 23	90	Acid Yellow 135	97
Disperse Yellow 54	91	Acid Yellow 151	98
Disperse Red 55	100	Acid Red 151	98
Disperse Red 60	100	Acid Red 337	100
Disperse Blue 7	98	Acid Orange 128	100
Disperse Blue 120	83	Acid Blue 25	95
		Acid Blue 40	92

* Disperse dyes were extracted with benzene, and acid dyes were immersed in pyridine - 2% NH₄OH (50:50) for one hour and then extracted with pure pyridine.

0 0 7 4

Table 18. Recovery Data of Previous Work*

Disperse Dyes	Recovery (%)	Acid Dyes	Recovery (%)
Disperse Yellow 3	98	Acid Yellow 19	70
Disperse Yellow 23	77	Acid Yellow 135	100
Disperse Yellow 54	77	Acid Yellow 151	75
Disperse Red 55	95	Acid Red 151	97
Disperse Red 60	89	Acid Red 337	75
Disperse Blue 7	73	Acid Orange 128	76
Disperse Blue 120	83	Acid Blue 25	80
		Acid Blue 40	84

* Disperse dyes were eluted with benzene and acid dyes were eluted with methanol and then with pyridine - THF - 1% NH₄OH (40:40:20).

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and are shown in Appendix B [27]. Structures of Direct Yellow 105 and Direct Yellow 106 have not been published although they are listed as stilbene derivatives.

Direct dyes contain large quantities of salts in addition to the dye. A purification procedure was required, therefore, to obtain samples for standard preparation. The technique used for purification [28] involved, first, dissolving the dye in DMF and filtering. The dye is soluble in DMF but the various inorganic salts present are not soluble. The dye is then recovered from DMF by addition of acetone to precipitate the dye. The precipitated dye is filtered, washed with acetone, and dried. This procedure was carried out twice to obtain purified dyes for preparation of standards.

Adsorption spectra of the six direct dyes selected for study are shown in Figures 11 and 12. A wavelength of 420 nm was selected for analysis for the yellow dyes, 520 nm for red dyes and 600 nm for blue dyes. It is clear from the spectra in Figures 11 and 12 that some absorbance from the red dyes will occur at 420 nm. Therefore, possible interference of the red dyes with analysis of the yellow dyes was expected. Similar problems should not be encountered in analysis for the direct red and direct blue dyes.

The structures of acid and direct dyes are similar in that both classes of dyes contain one or more ionic sodium sulphonate groups. It was expected, therefore, that the paired ion chromatograph (PIC) technique used to separate acid dyes could also be used for direct dyes. Initial experiments conducted by injecting solutions of direct dyes in 99% methanol-1% DMF in the liquid chromatograph (equipped with a C-18 bonded column) and eluting with a

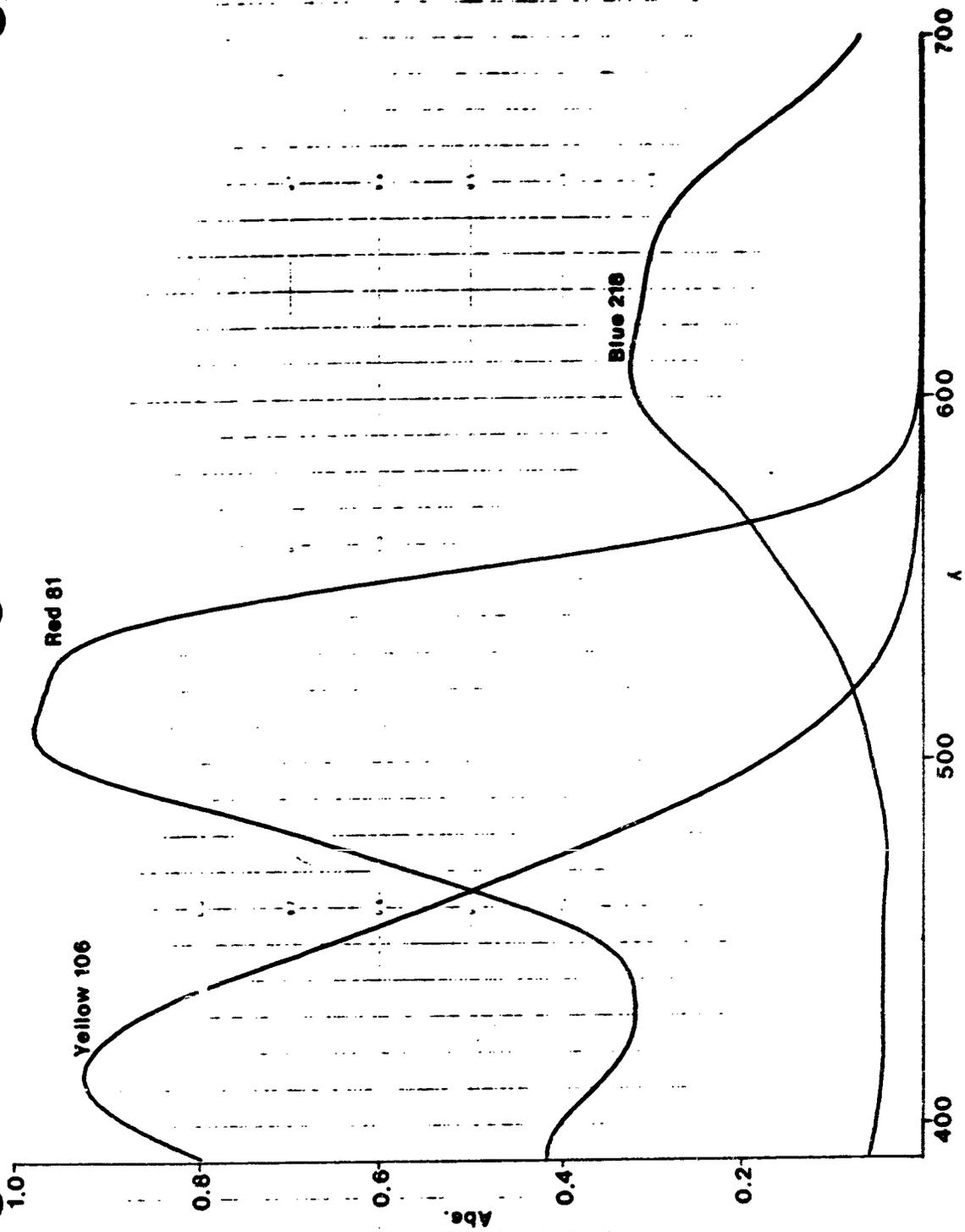


Fig. 11. Spectrophotometric Curves of Direct Yellow 106, Direct Red 81 and Direct Blue 218

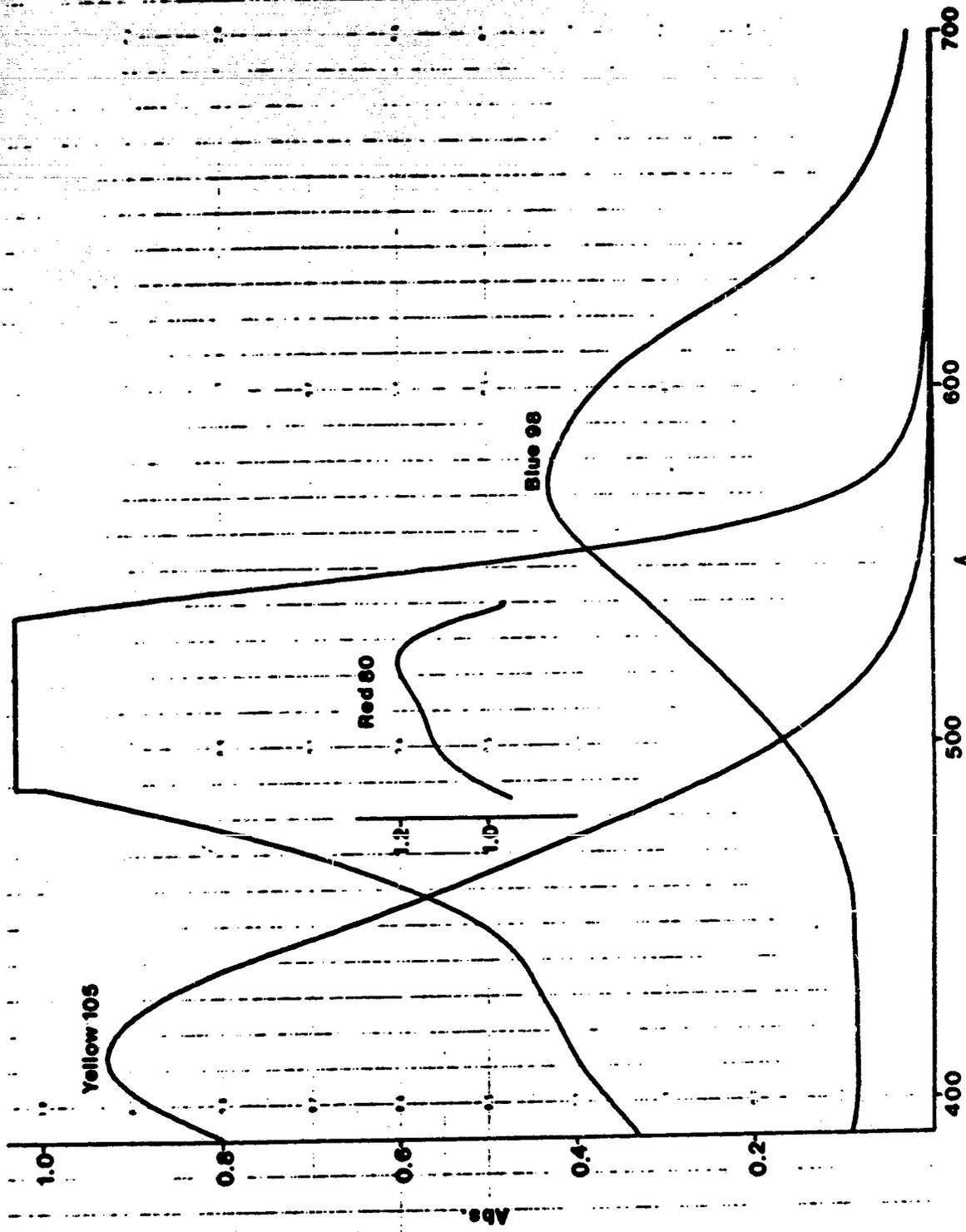


Fig. 12. Spectrophotometric Curves of Direct Yellow 105, Direct Red 80 and Direct Blue 94

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methanol water solvent mixture containing tetrabutylammonium phosphate confirmed this expectation. A chromatogram of a dye mixture containing 5 ppm each of Direct Yellow 106, Direct Yellow 105, Direct Red 80 and Direct Red 81 is shown in Figure 13. This chromatogram was obtained by a linear gradient elution beginning at 50/50 methanol/water and concluding at 85/15 methanol/water. The gradient time was 30 minutes at a total flow rate of 1 ml per minute. The detector was set at 420 nm. Both the direct yellow dyes are clearly separated and could be readily quantitated. It is interesting to note that Direct Yellow 105 gives two very distinct peaks in the chromatogram. Similar multiple peaks were found for most of the direct dyes.

A similar chromatogram of Direct Red 80 and Direct Red 81 is shown in Figure 14. The same gradient as above was run over a 10 minute period with the detector set at 520 nm. The two peaks from Direct Red 80 are clearly separated from Direct Red 81 under these conditions. It should be noted that the two peaks marked with X's in the chromatogram are artifacts due to a malfunction of the injector during this run.

Studies on Direct Blue 98 and Direct Blue 218 have shown that peaks from the dyes are separated very well with a 10 minute gradient from 50/50, methanol/water, to 85/15, methanol/water.

Thus, results of the liquid chromatography studies show that paired ion chromatography is a very effective technique for separating and quantitating direct dyes.

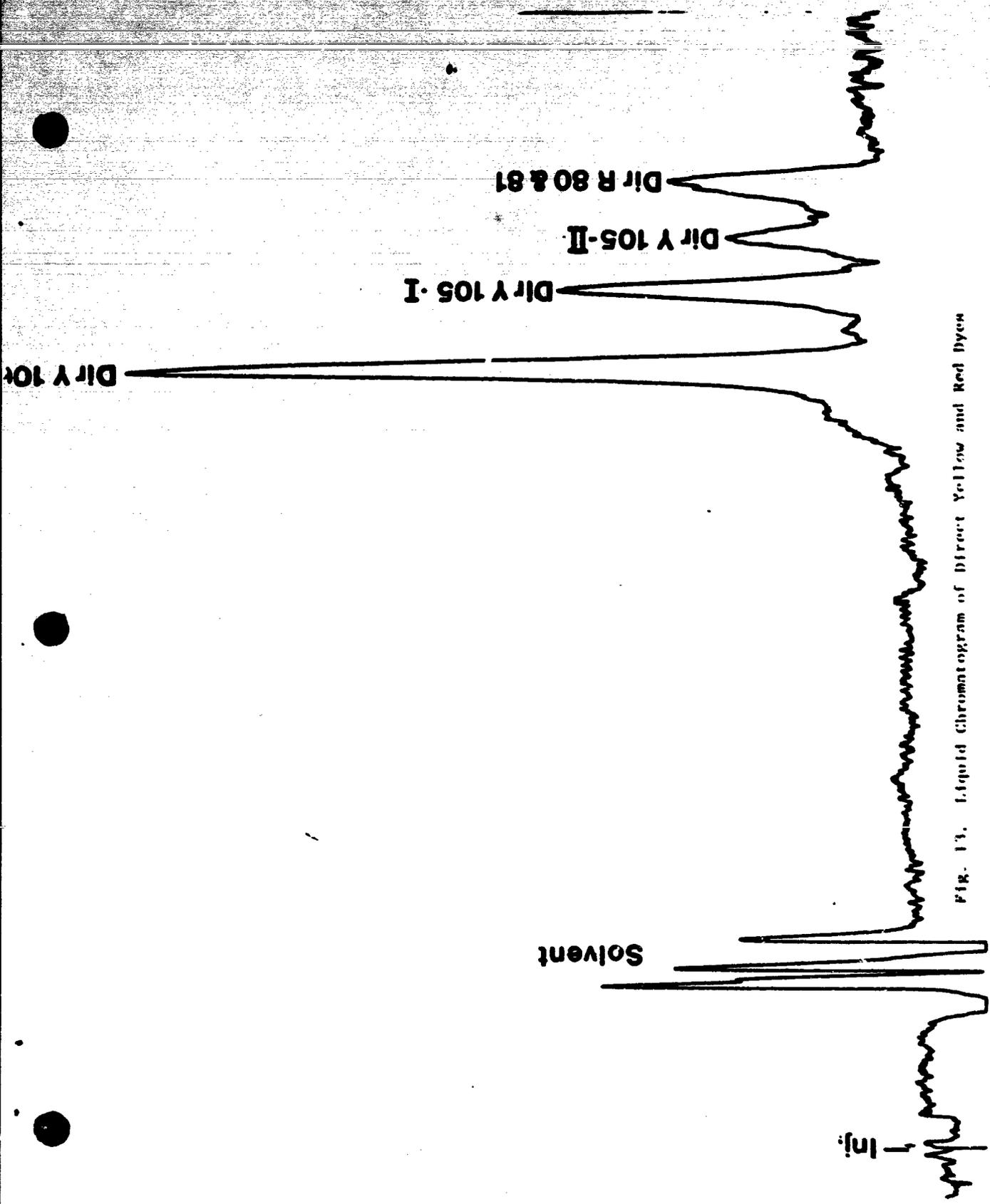


Fig. 13. Liquid Chromatogram of Direct Yellow and Red Dyes

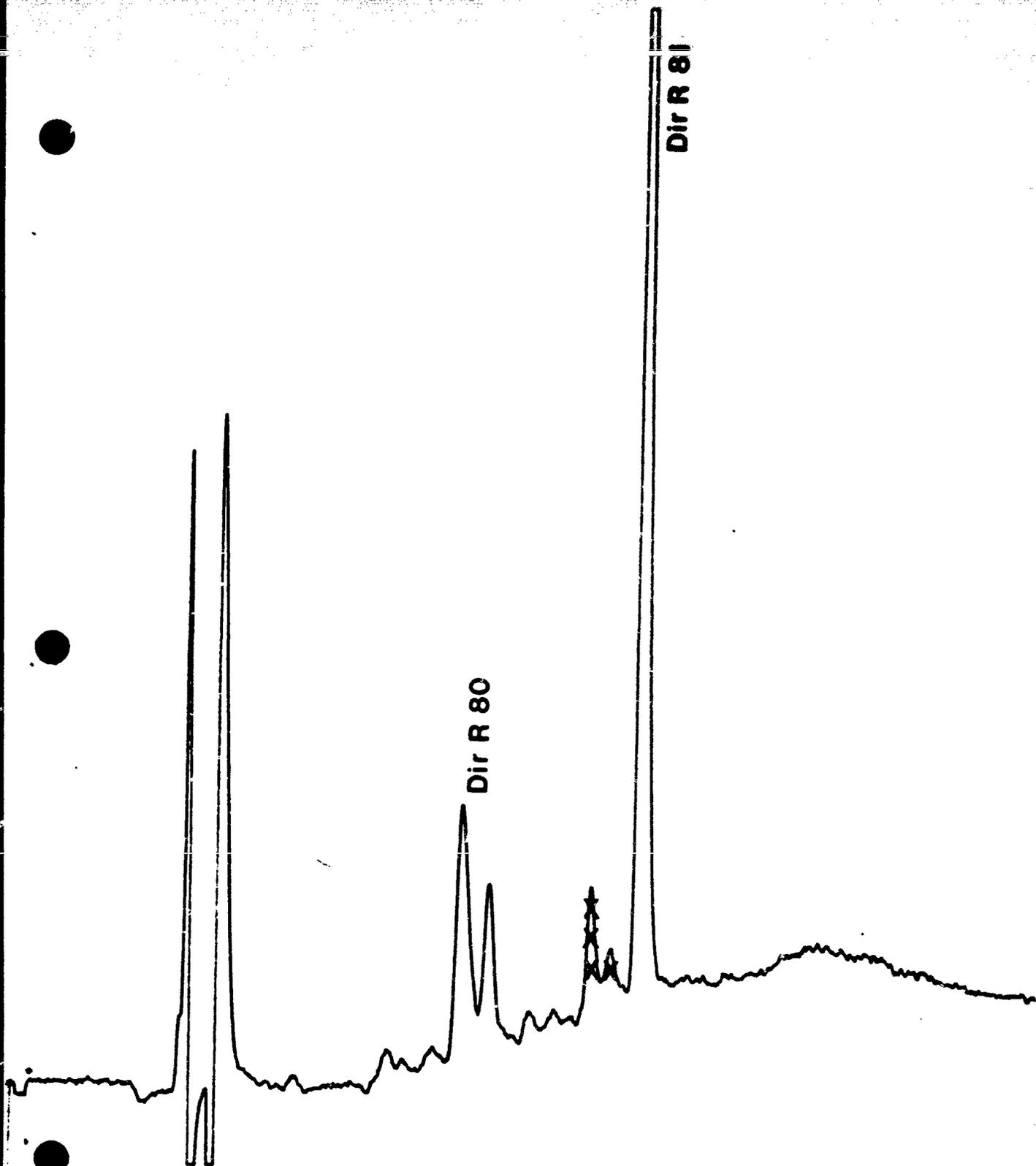


Fig. 14. Liquid Chromatogram of Direct Red Dyes

Preliminary experiments were also carried out to investigate the removal and concentration of direct dyes from wastewater. These experiments indicate that direct dyes can be removed from water by XAD-2 macroreticular resins. They are also removed from the resin by Soxhlet extraction with methanol. Thus, concentration procedures similar to those used for acid dyes should be applicable to direct dyes. Limitations in time and funds did not permit a complete study of the concentration phase.

Since acid dyes and direct dyes will be removed from the XAD resin columns by the same solvents, some experiments were carried out to determine if the acid and direct dyes could be separated and quantitated on the liquid chromatograph. A mixture of Direct Yellow 105, Direct Yellow 106, Direct Red 80, Direct Red 81, Acid Yellow 19, Acid Yellow 151 and Acid Yellow 135 were injected into the Chromatograph and a gradient run beginning at 55/45, and concluding at 85/15, methanol/water over a 30 minute period gave the chromatograph shown in Figure 15. Under these conditions Direct Yellow 106 begins to split into two components. The only overlap is the Acid Yellow 19 peak with one of the Direct Yellow 105 peaks. By determining the concentration of the Direct Yellow 105 from the second peak and correcting the area of the overlap peak for the Direct Yellow 105 peak 1, all 5 acid and direct yellow dyes can be quantitated.

In conclusion, the preliminary results suggest that the resin concentration-liquid chromatography system can be readily applied to analysis for direct dyes in wastewater. Further development of the system should make quantitative analysis of dyes in cotton and cotton polyester blend dyeing and in paper coloration wastewater a reality.

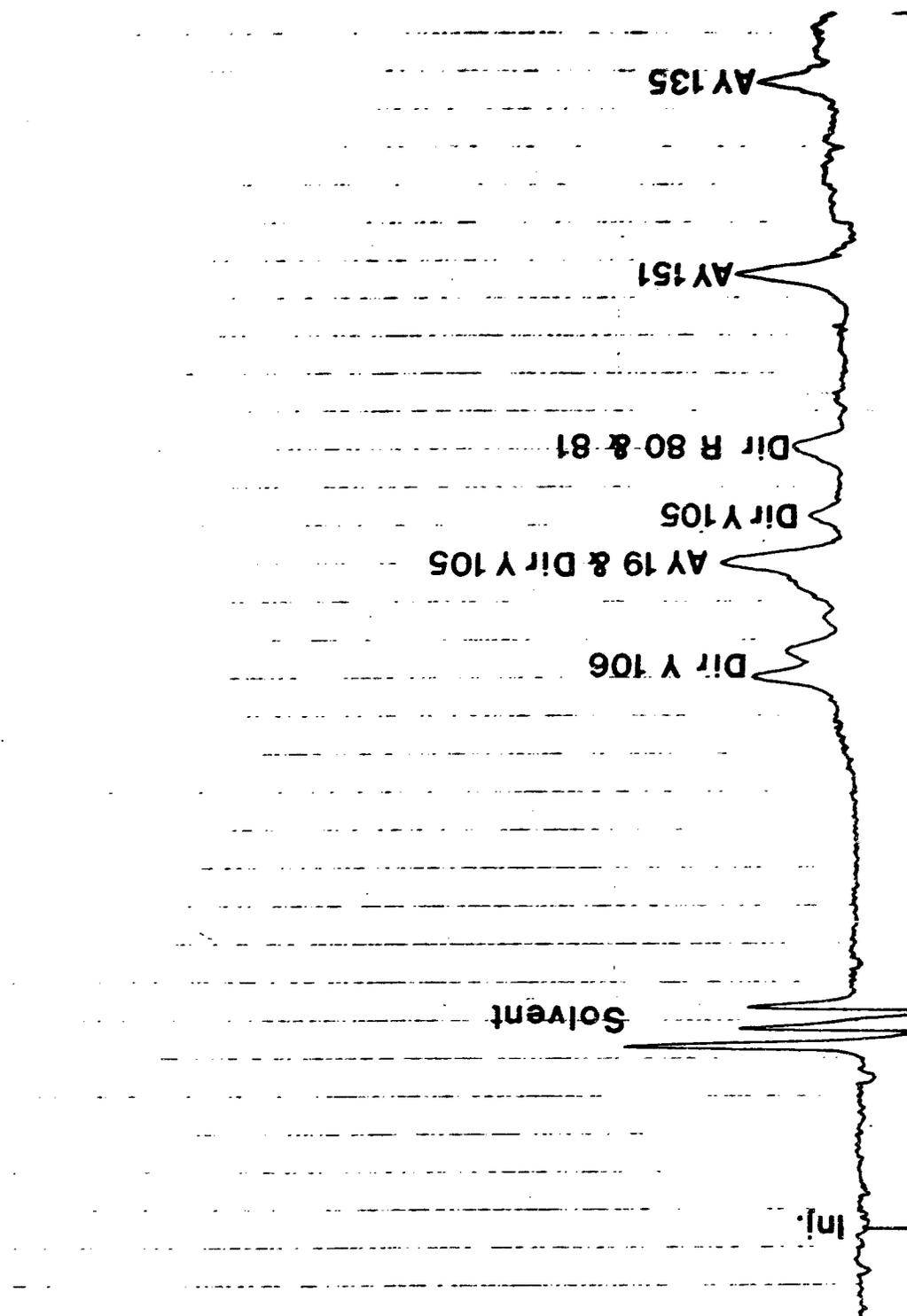


Fig. 15. Liquid Chromatograph of a Mixture of Direct Yellow, Direct Red and Acid Yellow Dyes

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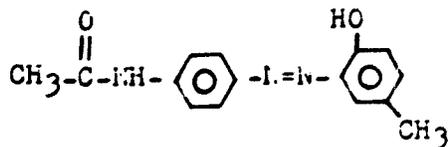
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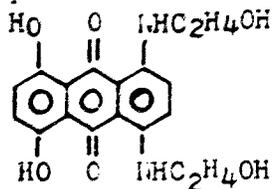
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Appendix A

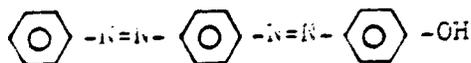
Disperse Yellow 3



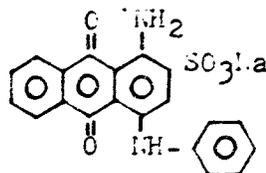
Disperse Blue 7



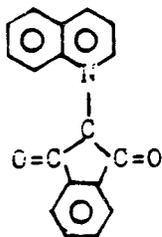
Disperse Yellow 23



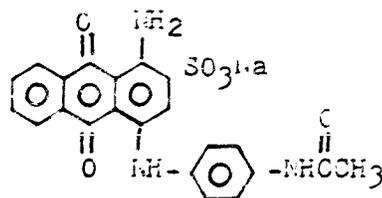
Acid Blue 25



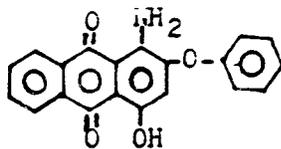
Disperse Yellow 54



Acid Blue 40

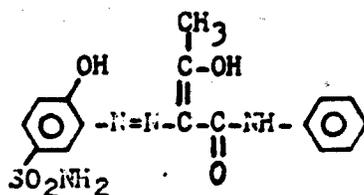


Disperse Red 60

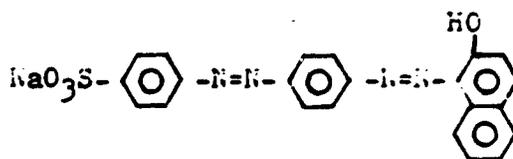


Appendix A (Cont'd.)

Acid Yellow 151



Acid Red 151



Appendix B. Structures of Direct Dyes

35780 C.I. Direct Red 80 (bright bluish pink)



(a) 6-Amino-1,4-azobenzene sulfonic acid -- N-Acetyl J acid;
then hydrolysis the acetamide group and phosgenate, or

(b) 6-Amino-1,4-azobenzene sulfonic acid (2 mol)
= 9,6-Diazenobis(1-naphthyl) sulfonic acid

Discovery - J. P. Penny 1922
National Aniline TSP 1590412
FIAT 764 - Strassrot FAB

Soluble in water (bluish red to magenta)
Very slightly soluble in ethanol and Cellosolve
Insoluble in other organic solvents
H₂SO₄ conc. = blue, on dilution = bluish red to violet
HNO₃ conc. = blue
Aqueous solution + HCl conc. = violet ppt.
NaOH conc. = reddish violet

28160 C.I. Direct Red 81 (Bright red)



p,p'-Aminophenylazobenzene sulfonic acid + N-Benzoyl J acid

Aqueous solution + HCl conc. = violet bluish precipitate
NaOH conc. = reddish violet

Discovery - E. Hesse, G. Gunther and A. Zart 1909
Bayer Co. BP 476800 TSP 931423 + EP 4 02126; GP 12
E23375 (Fr. 10 825)
BIOB 1548-160
FIAT 764 - Strassrot FAB
Wannert, Z. anorg. Chem. 36 (1925), 511

Soluble in water (bright red to magenta)
Slightly soluble in ethanol
Insoluble in other organic solvents
H₂SO₄ conc. = reddish blue precipitate
HNO₃ conc. = reddish blue precipitate

23155 C.I. Direct Blue 98 (Blue)

Blue Copper compound, derived from



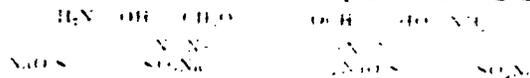
1-Naphthol-3-sulfonic acid +
N-Glycyl J acid

Can make an intimate mixture of the two dyes, the mixture reacts with copper sulfate and sodium acetate by means of a diazotization reaction at 120-125°C. The diazotization reaction is aided by microwave comp. during the process.

Discovery - R. Hesse, 1911
IG., BP 32956; TSP 188975; GP 1150
E12, 18, 19, 20
BIOB 1548-152
FIAT 764 - Strassrot FAB

Soluble in water (reddish blue)
Very slightly soluble in ethanol
H₂SO₄ conc. = greenish blue on dilution = reddish blue
Aqueous solution + HCl conc. = reddish blue ppt.
NaOH conc. = reddish blue ppt.

24400 C.I. Direct Blue 15 (Blue) (Blue 218 with Cu)



1-Diaminobenzene + 1,4-Diaminobenzene

Aqueous solution + HCl conc. = reddish blue ppt.
NaOH conc. = violet ppt.

Discovery - J. B. Bauman, A. C. Clark and M. H. G. 1906
Cassella Co. BP 374, 375; TSP 362135; GP 574; GP 580
EP 204770; GP 771; GP 3 684
BIOB 1548-173
FIAT 764 - Strassrot FAB

Soluble in water (reddish blue)
Insoluble in other organic solvents
H₂SO₄ conc. = bluish green on dilution = reddish blue
HNO₃ conc. = reddish blue precipitate

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APPENDIX C

Analytical Procedure for Analysis of Dyes in Wastewater

Scope: This method is applicable to the determination of 15 acid and disperse dyes (Acid Yellow 19, Acid Yellow 135, Acid Yellow 151, Acid Orange 128, Acid Red 151, Acid Red 337, Acid Blue 25, Acid Blue 40, Disperse Yellow 3, Disperse Yellow 23, Disperse Yellow 54, Disperse Red 55, Disperse Red 60, Disperse Blue 7, Disperse Blue 120) in wastewater.

Apparatus: Chromatography Columns (9 mm x 500 mm) equipped with demountable stopcocks and reservoirs. Rotary vacuum evaporator. High-pressure liquid chromatograph equipped with variable wavelength UV-visible detector and solvent gradient capability. Soxhlet extractor (large).

Chemicals: Liquid Chromatography Grade Dimethylformamide
Liquid Chromatography Grade Cyclohexane
Liquid Chromatography Grade Tetrahydrofuran
Liquid Chromatography Grade Methanol
Liquid Chromatography Grade Benzene
High Purity Distilled Water
PIC Reagent A (Tetrabutyl ammonium phosphate)--Waters Associates
Dye Samples for standard preparation

Purified Dye Samples: Disperse Dyes--Place 50 grams of disperse dye in a glass extraction thimble (course fritted disk). Extract in Soxhlet extraction unit until extract is clear. Evaporate extract in a rotary vacuum evaporator until dry. Repeat the extraction on the recovered dye. Place the reserved pure dye from the second extraction in a tightly closed container and place in a dessicator.

Acid Dyes--Dissolve 50 grams of acid dye in 300 ml of dimethylformamide. Filter and retain the filtrate. Precipitate the dye from the dimethylformamide by the addition of 300 ml of acetone. Recover the dye by filtration. Repeat the purification. Place the pure dye recovered from the last filtration in a tightly closed container and place in a dessicator.

Standard Solutions: Weigh 1.00000 gram of purified disperse dye and transfer to a 1 liter volumetric flask. Add 10 ml of dimethylformamide (LC grade) and fill to calibration mark with benzene (LC grade) for a 1 gram per liter standard stock solution. For standard acid dye solutions, weigh 1.00000 gram of purified acid dye and transfer to a 1 liter volumetric flask. Add 10 ml of dimethylformamide (LC grade) and fill to calibration mark with methanol (LC grade). Prepare 5, 10 and 20 ppm solutions of dyes by pipetting the appropriate quantity of stock solution into a 100 ml volumetric flask and diluting to the calibration mark with 1% DMF/99% benzene (LC grade) for disperse dyes or 1% DMF/99% methanol (LC grade) for acid dyes.

Wastewater Samples: Wastewater samples should be collected in clean glass bottles and kept at 0°C until concentrated.

Resin Column Preparation: Place approximately 50 cc of Amberlite XAD-2 macroreticular r-sin (Rohm and Haas Company) in a glass Soxhlet extraction thimble. Extract with 250 ml of methanol for 2 hours. Discard the methanol and extract with benzene for 2 hours. Discard the benzene and fill the extraction thimble with a 50:50 pyridine:2% ammonium hydroxide solvent mixture. Allow to stand for one hour then extract for 2 hours with pyridine. Discard the pyridine/ammonium hydroxide solution and dry the purified resin in a warm (75°C) oven. Slurry the purified resin in 100 ml of methanol. Pour the slurry into a prepared chromatography column allowing the methanol to drain through the stopcock. Collect approximately 30 ml of resin in the column. Downflow wash the resin with 200 ml of water followed by an upflow wash of 40 ml of water to reclassify the column. Store the column under distilled water until ready for use.

Dye Concentration: Take 1800 ml of the wastewater sample and add 200 ml of DMF. Divide the sample in two approximately equal portions and pass the solution through two prepared XAD-2 resin columns at 4 bed volumes (120 ml) per hour. Rinse the column with 40 ml of distilled water. Drain as much water from the column as possible then transfer the resin from both columns into a single large extraction thimble. Dry the resin-containing dye in a warm (75°C) oven overnight. Place the extraction thimble in a soxhlet extractor and extract with benzene until the extract is clear (approximately 4 hours). Place the extract in a rotary evaporator and evaporate to dryness. Take the extracted dye up in 10 ml of a 1% DMF/99% benzene solvent mixture. After extraction of the resin with benzene place the resin and thimble in the drying oven to remove residual benzene. Return the dry resin to the Soxhlet extractor and fill the thimble with a 50:50, pyridine:2% ammonium hydroxide solvent mixture. Allow to stand for 1 hour and then extract with pyridine until the extract is clear (approximately 4 hours). Place the extract in the rotary evaporator and evaporate to dryness. Take the residue up in 10 ml of a 1% DMF/99% methanol solvent mixture.

Analysis for Disperse Dyes: Place a 25 cm column with cyanoethyl groups bonded to a silica substrate (Partisil 10-PAC from Whatman or equivalent) in the liquid chromatograph. All analyses are run with a 1 ml/minute solvent flow rate and with a 20 microliter sample size.

Red 55--Equilibrate the column by flowing 35/65 mixture of tetrahydrofuran/cyclohexane through the column for 15 minutes. Monitor the effluent at 520 nm. Inject samples of the 5, 10 and 20 ppm standard solutions of Disperse Red 55 sequentially into the chromatograph. The dye peak appears just after the solvent front (see Figure 3). Prepare a plot of area under the curve versus concentration. Inject a sample of the concentrated disperse dye mixture. Determine the area of the peak from Disperse Red 55. Determine the concentration of Disperse Red 55 from the calibration curve. Strip other dyes from the column by passing 100% tetrahydrofuran through the column for 15 minutes.

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Red 60--Equilibrate the column by flowing a 80/20 tetrahydrofuran/cyclohexane solution through the column for 15 minutes. Monitor the effluent at 520 nm. Inject samples of the 5, 10 and 20 ppm standard solution of Disperse Red 60 sequentially into the chromatograph. The dye peak will appear just after the solvent front. Prepare a plot of area under the peak versus concentration. Inject a sample of the concentrated disperse dye mixture. Determine the area under the peak from Disperse Red 60. Determine the concentration of Disperse Red 60 from the calibration curve. Strip other dyes from the column by passing 100% THF through the column for 15 minutes.

Blue 120--Equilibrate the column by flowing a 45/55 tetrahydrofuran/cyclohexane mixture through the column for 15 minutes. Monitor the effluent at 620 nm. Inject samples of the 5, 10 and 20 ppm standard solution of Disperse Blue 120 sequentially into the chromatograph. The dye peak will appear just after the solvent front. Prepare a plot of area under the peak versus concentration. Inject a sample of the concentrated disperse dye mixture. Determine the area under the peak from Disperse Blue 120. Determine the concentration of Disperse Blue 120 from the calibration curve. Strip other dyes from the column by passing 100% THF through the column for 15 minutes.

Blue 7--Equilibrate the column by passing 100% THF through the column for 15 minutes. Monitor the effluent at 620 nm. Inject samples of the 5, 10 and 20 ppm standard solutions of Disperse Blue 7 sequentially into the chromatograph. Several peaks will appear with two principal peaks at approximately 2 and 4 minutes retention time (see Figure 2). Determine the area of either of the two major peaks and plot area under the peak versus concentration. Inject a sample of the concentrated disperse dye mixture. Determine the area under the selected peak and obtain the concentration of Disperse Blue 7 from the calibration curve.

Yellow Dyes--The three yellow disperse dyes can be determined from a single sample injection. The column is equilibrated by pumping a 25/75 mixture of tetrahydrofuran/cyclohexane through the column for 15 minutes. The effluent is monitored at 420 nm. A 20 microliter sample of 20 ppm of each of the yellow dyes is injected and a solvent gradient varying linearly from 25/75 to 100/0 tetrahydrofuran/cyclohexane is run over 15 minutes. Disperse Yellow 54 elutes first followed by Disperse Yellow 23 with Disperse Yellow 5 eluting last (see Figure 1). Reset the gradient controller to 25/75 tetrahydrofuran/cyclohexane. Repeat the calibration procedure by injecting standard solution containing 10 ppm of each disperse yellow dye and 5 ppm of each disperse yellow dye. Plot areas under the peaks versus concentration for each of the yellow disperse dyes. Inject a 20 microliter sample of the concentrated disperse dye mixture and elute with the solvent gradient. Determine the areas under the peaks eluting at the same times as the standards and calculate the concentration from the areas using the calibration curves.

Analysis for Acid Dyes: Place a 25 cm 5 micron silica with C-18 hydrocarbon bonded to the surface column (e.g., Spherisorb ODS, Laboratory Data Control) in the liquid chromatograph. Prepare the elution solvents by dissolving 1 vial of buffered tetrabutylammonium phosphate (PIC Reagent A, Waters Associates) in methanol (LC grade) and 1 vial in high purity distilled water. Filter the solvents to remove any particulates from the PIC A reagent. Prepare a 60/40, methanol/water, solvent mixture and an 85/15, methanol/water, mixture. The 60/40 mixture is used as solvent A in the gradient system and the 85/15 mixture as solvent B. Filter both solvents A and B and heat the filtrate for 1 minute at the boil under reflux to degress the solvents. A flowrate of 1 ml per minute, a solvent gradient of 0% A to 100% B over a 10 minute period is employed in all acid dye analyses. A flowrate of 1 ml per minute is used.

Acid Blue Dyes--Set the detector at 615 nm. Inject a solvent blank (20 microliters of a 1% DMF/99% methanol solvent mixture) and run the elution gradient. This run will serve for background calibration. Reset the gradient programmer to 0% A. Inject a 20 microliter sample of a 20 ppm mixture of Acid Blue 40 and Acid Blue 25. Two major peaks will be observed with the Acid Blue 40 peak appearing first (see Figure 6). Run a 10 ppm mixture and a 5 ppm mixture of each of the Acid Blue dyes in a similar manner. Prepare a plot of peak area versus concentration for each of the Acid Blue dyes. Inject a 20 microliter sample of the dye concentrate from the wastewater and elute in the same manner as the standards. Determine the area under the peaks eluting at the same time as Acid Blue 40 and Acid Blue 25 and determine their concentration from the calibration curves.

Acid Red and Orange Dyes--Set the detector at 520 nm. Inject a solvent blank (20 microliters of a 1% DMF/99% methanol solvent mixture) and run the elution gradient for background calibration. Reset the gradient programmer to 0% A. Run a 20 ppm, 10 ppm and 5 ppm standard mixture of Acid Red 337, Acid Orange 128 and Acid Red 151, sequentially. The Acid Red 337 peak appears first, the Acid Orange 128 second and the Acid Red 151 last (see Figure 5). Prepare calibration curves with peak area plotted against concentration for each of these dyes. Inject a 20 microliter sample of the dye concentrate from the wastewater and elute in the same manner as the standards. Determine the areas under the peaks eluting at the same time as peaks in the chromatograms of the standards and determine the concentrations from the calibration curves.

Acid Yellow Dyes--Set the detector at 420 nm: Inject a solvent blank (20 microliters of a 1% DMF/99% methanol solvent mixture) and run the elution gradient for background calibration. Reset the gradient program to 0% A. Run a 20 ppm, 10 ppm and 5 ppm standard mixture of Acid Yellow 19, Acid Yellow 151 and Acid Yellow 135, sequentially. If Acid Orange 128 was present in the wastewater sample (see above) run 20 ppm, 10 ppm and 5 ppm standard samples of Acid Orange 128. The Acid Yellow 19 peak appears first, the Acid Yellow 151 peak next and the Acid Yellow 135 peak appears last (see Figure 4). The Acid Orange 128 peak elutes at the same retention time as Acid Yellow 151. Prepare calibration curves of peak area versus concentration for the Acid Yellow and Acid Orange dyes. Inject a 20 microliter sample of the dye concentrate from the wastewater and elute in the same manner as the stan-

dard dye solutions. Determine the areas under the peaks eluting at the same retention time as peaks in the chromatograms of the standards. Calculate the concentrations of Acid Yellow 19 and Acid Yellow 135 from the calibration curves. Using the Acid Orange 128 calibration curve calculate the area of the peak expected at 420 nm from the concentration of Acid Orange 128 found previously. Subtract this area from the second peak (combined Acid Orange 128 and Acid Yellow 158). Determine the concentration of Acid Yellow 151 from the remainder using the appropriate calibration curve.

Sensitivity: All dyes except Disperse Blue 7 and Disperse Blue 120 can be detected at 1 part-per-billion in wastewater. Acid Blue 7 can be detected at 10 parts-per-billion and Acid Blue 120 at 25 parts-per-billion.

Other Applications: The analytical system can be used for solid (mud) samples by extracting the dry mud in exactly the manner described for the Soxhlet extraction of the resin in the dye concentration phase. A total of 1,000 grams of dry mud should be extracted for subsequent analysis.

THE DEGRADATION OF DYESTUFFS: PART II
BEHAVIOUR OF DYESTUFFS IN AEROBIC BIODEGRADATION TESTS

U. Pagga and D. Brown*

ABSTRACT

Eighty-seven dyestuffs have been tested in short-term aerobic biodegradation tests. The results confirmed that dyestuffs are most unlikely to show any significant biodegradation in such tests. With many dyestuffs a substantial colour removal was observed which may be attributed to the elimination of the dyes by adsorption. In some cases DOC (Dissolved Organic Carbon) removal did not correlate with colour removal and this is attributed to the presence of non-coloured organic components in the dyestuff.

INTRODUCTION

Commercial dyestuffs all derive their colour from the relatively complex chromophore system which they contain. A necessary criterion for their successful use is that they shall be stable in light and washing processes, and also stable against microbial attack. For these reasons it cannot be expected that dyestuffs, in general, will give positive results in short-term tests for aerobic biodegradability. Notwithstanding this, there are few reports of dyestuffs being detected in the environment at large (1,2) and thus it seems probable that some physico-chemical or biodegradative removal processes are operating.

The aim of this present work, as part of the ETAD** continuing programme of investigations into the environmental fate of dyestuffs (3,4), was to investigate whether some dyestuffs might be susceptible to aerobic biological degradation and, if so, to what extent this occurs. Previous studies on the aerobic degradation of dyestuffs have used relatively simple dyestuff model compounds (5,6) and highly adapted bacteria, but in this present work the dyestuffs chosen were typical commercial products and the bacterial inocula were from wastewater treatment plants.

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MATERIAL AND METHODS

Readily water-soluble dyestuffs were selected for this test programme on the basis that they were less likely to be adsorbed to a significant extent onto activated sludge. Some materials were tested as the technically pure substance, the so-called "press-cake", and others were tested as sales products which may contain other organic substances such as wetting agents, de-dusting agents, etc.

In the first stage of the investigation, 87 different dyestuffs were investigated using a static test with activated sludge as the inoculum. From this first stage 5 of the dyestuffs which had given results indicating that they were possibly biodegradable were selected for further tests. These further tests were designed to show whether or not they were genuinely biodegradable or simply being removed by adsorption onto the activated sludge.

Static test with activated sludge

A modification of the OECD Guideline 302B (7) was used as the static test method. This procedure provides a rather simple simulation of the conditions of an adapted activated sludge waste water treatment plant, and is generally considered to provide an indication of the inherent biodegradability of the test substance. In common with most sewage works simulation tests it cannot in all cases distinguish between true biodegradation and bioelimination due to flocculation or adsorption onto the activated sludge. However, if an analysis carried out within the first day of the test (normally 3 hours) indicates significant elimination, it is presumed that this must be due to physical processes rather than biodegradation. It remains an open question whether a substance so removed by a physical process is then subsequently biodegraded.

The modifications of the method used in this present work were:

- concentration of dyestuff 100 mg/l
- concentration of activated sludge 0.5 g/l dry material
- feeding of the inoculum each week 100 mg/l yeast extract (in most cases)
- test duration up to 42 days
- analytical methods extinction at absorption maximum and DOC (dissolved organic carbon)
- initial DOC concentration calculated from stock solution measurement

The main variations from the OECD method were the extension of the test period from 28 to 42 days and, because the test time was relatively long, the weekly feeding of the sludge by most of the participating laboratories.

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The sources of the activated sludge were treatment plants conveniently located to the laboratories carrying out the test. These treatment plants received communal and/or industrial wastewater.

Modified OECD screening test

This test was performed essentially according to the OECD Guideline 301E (8). The DOC concentration of the test substances was between 20 and 40 mg/l and the test duration was between 21 and 49 days. As in the static test the criteria for biodegradation were both decolourisation at the absorption maximum and DOC elimination. The inoculum source was treated effluent from municipal sewage treatment plants receiving predominantly domestic wastewater.

Some additional experiments were also carried out using an inoculum which has had the opportunity to become adapted to the dyestuff. The adaptation procedure was to incubate the dyestuff with activated sludge in a static test (as mentioned above) and in one case in a modified SCAS (Semi-Continuous Activated Sludge) test (9). The supernatant from these tests was then used to inoculate the OECD screening test.

Respirometric test

The method used was based on the OECD Guideline 301C (10) using as inoculum activated sludge from the same wastewater treatment plants as used for the static test. This type of procedure and its development as a method for the assessment of "ready" biodegradability has been described by Painter and King (11).

In this present work the concentration of the dyestuff was in most cases 100 mg/l and the test duration was 28 days. The main criterion for biodegradation was the Biological Oxygen Demand (BOD) as measured during the test, expressed as a percentage of the Chemical Oxygen Demand (COD). Colour and DOC removal were also in some cases measured at the end of the test period.

RESULTS AND DISCUSSION

The criteria for the classification of the static test results are shown in Table 1. These criteria have been used to classify the results of this test (Table 3), both in terms of colour and of dissolved organic carbon (DOC) removed. For those dyestuffs (44) where there is agreement between colour and DOC removal, an overall classification using the Table 1 criteria was also made. These results are summarised in Table 2 and in Figure 1 (Elimination of Colour) and Figure 2 (Elimination of DOC).

Table 1 Limit values for classification of static test results

Category	Classification	Percentage of elimination (colour and/or DOC)	
		after 3 hours (%)	Test end (%)
A	Possibly biodegradable	< 25	> 70
B	Partial elimination - biodegradation processes possible	< 25	25 - 70
C	Probably elimination mainly by adsorption onto the sludge	> 25	> 25
D	No elimination in the static test	< 25	< 25

Table 2 Summary of results of the static test

Category	only decolourisation		only DOC elimination		Decolourisation and DOC elimination	
	Number	%	Number	%	Number	%
A Possibly biodegradable	11	13	11	13	7	8
B Partial elimination	23	26	16	18	4	5
C Adsorption	20	23	13	15	11	13
D No elimination	33	38	47	54	22	25
Not classifiable (Colour and DOC differ)	-	-	-	-	43	49
Total number of dyestuffs	87	100	87	100	87	100

As indicated above, only about half (44 out of 87) of the dyestuffs tested gave results for DOC and colour removal which were in broad agreement. The main reason for this apparent anomaly is likely to have been a difference in the removal levels of the coloured component of the dyestuff, and the non-coloured organic material which may be present in significant quantities in commercial dyestuffs.

Of the dyestuffs placed in category D (elimination less than 25% at the end of test), Figure 1 shows that 38% were placed there using colour as the criterion, though using DOC (Fig. 2) slightly over half of the dyes tested did not give any appreciable removal.

For the dyestuffs placed in category C (immediate elimination by adsorption) 23% were placed there on the colour criterion and 15% on DOC. Since the dyes for this study were selected on the basis that they were less likely to be removed by adsorption onto the sludge, it may be expected that in a random selection of dyestuffs, the number in this group would have been higher. It should also be noted that adsorbed dyestuffs may subsequently be biodegraded, but the static test does not give sufficient information to distinguish the process.

Of the dyestuffs in the partial elimination category B (< 25% immediate elimination, 25-70% by the end of the test), 26% of the dyes tested were placed there on the colour criterion and 18% on DOC, but for only 5% of the 87 dyes were colour and DOC in agreement.

In the possibly biodegradable category A (< 25% immediate elimination, > 70% by the end of test), 13% of the dyes were placed in this category using colour or DOC as the criterion, and seven dyes (8% of the total) were placed in this category on the basis of both colour and DOC. Of these seven dyes, five were selected for further testing by the modified OECD screening test and also by a respirometric test.

The results of the modified OECD screening test (Table 4) generally show reasonable correlation between the removal of colour and of DOC, and that even with an adapted inoculum, no dyestuff showed any convincing evidence of genuine biodegradation.

The results of the respirometric tests are given in Table 5, and show some distinct anomalies, both between the same parameter as measured by different laboratories and between different parameters measured by a single laboratory on an individual dyestuff. The main parameter measured in this test was the biological oxygen demand (BOD) at the end of the test (usually 28 days) and this is expressed in the table either as mg O₂/g substance or as % BOD/COD. All of the dyes tested showed a positive BOD in at least one laboratory, though equally all of the dyes tested showed zero, or effectively zero, BOD in two or more other laboratories. Some laboratories also measured DOC and/or colour removal at the end of the respirometric test. In the case of colour (measured in 2 laboratories) no dyestuff showed a colour removal in excess of 20%. This could easily be due to adsorption of the dye over the test period. DOC removal was also measured in 2 laboratories (3 laboratories investigated Direct Yellow 44) but for this parameter the results were more variable, both for individual dyes and across the five dyestuffs.

The tentative conclusion from the above and from the results of the modified OECD screening test is that the five dyes tested in this second phase of the work probably contain some biodegradable component but that this is not the chromophore. Such decolourisation as was observed is probably due to sorption mechanisms.

Finally, as was noted under section 'Modified OECD screening test', one laboratory used the SCAS test in an attempt to produce an adapted inoculum for the modified OECD test. This SCAS

test was carried out for a period of 4 months using 5 dyestuffs selected for the OECD and respirometric studies. No colour removal was observed for any of these dyes and since the SCAS test is accepted as being a "forcing" test for inherent biodegradability, it indicates that the positive result found for these 5 dyes in the static test was almost certainly not genuine biodegradation.

CONCLUSIONS

The results of this work confirm that, as expected from their structures and function, dyestuffs are most unlikely to show as biodegradable in short-term aerobic tests for ready biodegradability, and there seems little point in carrying out such test procedures.

In a test such as the OECD Guideline 302B (static test) which may be regarded both as an investigation of inherent biodegradability and as a relatively simple screening test to assess likely removal during the biological treatment, a large number of dyestuffs (62% of those tested in this present work) are likely to show a significant removal of colour, indicating that such dyestuffs will be removed at least to some extent during biological treatment. This test is also likely to show elimination of DOC in a significant number of dyestuffs (46% of those tested in this work).

On balance, it seems likely that the prime mechanism for the removal of the coloured component of dyestuffs is by adsorption of the intact dye molecules. However, it has not been possible to demonstrate unequivocally that aerobic biodegradation processes either have, or have not, occurred in the chromophoric components of the dyes tested and for some dyestuffs it is possible that relatively minor biodegradative changes occur to render the dye more amenable to removal. Previous and current work by ETAD shows that under anaerobic sludge digestion conditions adsorbed dyestuffs are in general susceptible to degradation.

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Figure 1 Elimination of colour

Static test with activated sludge

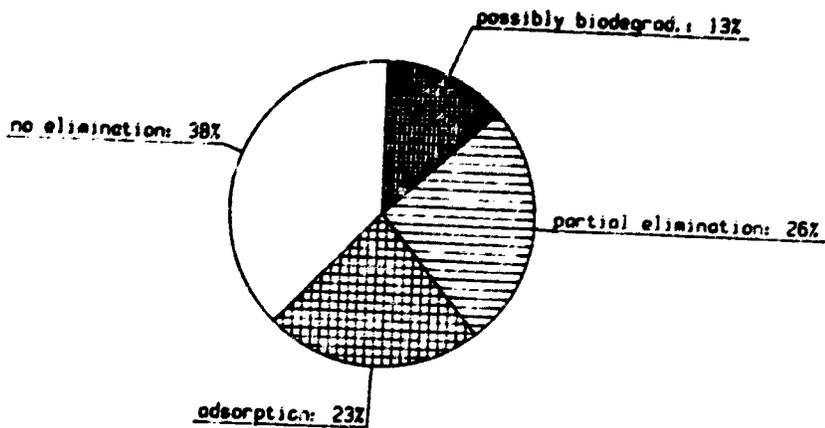


Figure 2 Elimination of dissolved organic carbon

Static test with activated sludge

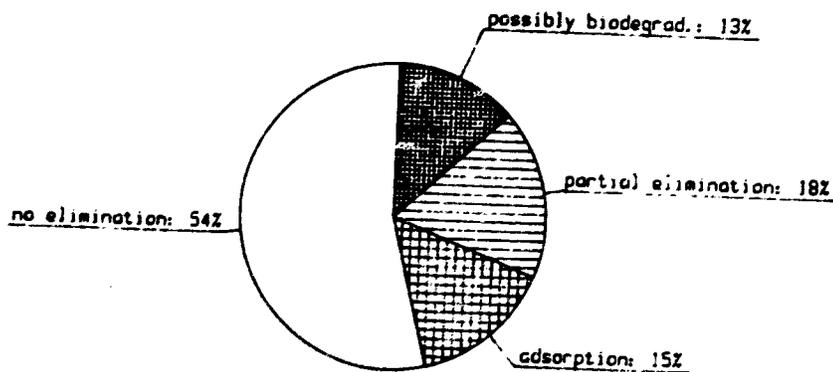


Table 3 Results of the static test with activated sludge

Nr	dye stuff	DOC start mg/l	DOC ads %	DOC elim %	DOC Cat	col ads %	col elim %	col cat	elim Cat	lambda nm	time d
1	Acid Black 1	23	-26	30	B	2	18	D	-	614	35
2	Acid Black 2	23	0	96	A	1	75	A	A	560	42
3	Acid Black 48	20	-25	00	A	5	61	B	-	646	42
4	Acid Black 84	25	12	36	B	3	20	D	-	600	42
5	Acid Blue 9	56	14	45	B	2	0	D	-	630	42
6	Acid Blue 9	20	35	33	C	33	33	C	C	615	24
7	Acid Blue 15	28	4	4	D	49	100	C	-	560	42
8	Acid Blue 41	80	16	84	A	14	84	A	A	590	24
9	Acid Blue 92	46	-5	10	D	0	30	B	-	566	42
10	Acid Blue 113	50	70	70	C	92	96	C	C	563	41
11	Acid Brown 100	20	72	20	D	72	75	C	-	450	24
12	Acid Brown 159	35	-9	74	A	21	26	A	A	460	42
13	Acid Brown 248	10	0	-200	D	0	100	A	-	424	42
14	Acid Brown 311	103	75	67	C	75	84	C	C	440	41
15	Acid Green 25	24	-29	29	B	0	14	D	-	408	42
16	Acid Green 108	43	7	28	B	8	43	B	B	645	42
17	Acid Orange 3	41	0	-29	D	-15	-18	D	D	360	30
18	Acid Orange 61	30	-3*	-23	D	54*	67	C	-	555	42
19	Acid Orange 67	42	69	76	C	68	99	C	C	437	24
20	Acid Red 18	96	0	5	D	0	5	D	D	502	40
21	Acid Red 73	32	-3	6	D	0	17	D	D	504	42
22	Acid Red 87	30	17	-3	D	0	7	D	D	520	42
23	Acid Red 97	12	-8	17	D	20	20	D	D	484	42
24	Acid Red 183	29	24	28	D	0	28	D	D	499	42
25	Acid Red 186	36	25	6	D	1	0	D	D	545	42
26	Acid Red 214	6	33	-167	D	3	0	D	D	495	42
27	Acid Red 348	76	97	93	C	98	98	C	C	514	15
28	Acid Red 413		19	15	D	9	7	D	D	515	42
29	Acid Red 413		86	97	C	83	83	C	C	525	42

Table 3 continued

Nr	dye stuff	DOC start mg/l	DOC ads %	DOC elim %	DOC cat	col ads %	col elim %	col cat	elim cat	lambda nm	time d
30	Acid Yellow 3	34	-98	-44	D	2	0	D	D	416	42
31	Acid Yellow 36	53	15	11	D	0	5	D	D	440	42
32	Acid Yellow 54	29	-3	0	D	3	16	D	D	440	42
33	Acid Yellow 61	69	73	85	C	75	95	C	C	400	42
34	Acid Yellow 73	63	18	11	D	1	5	D	D	490	42
35	Acid Yellow 151		20	26	D	25	26	D	D	440	42
36	Acid Yellow 176	19	63	32	C	0	2	D	-	410	42
37	Acid Yellow 237	37	27	49	B	34	18	D	-	450	42
38	Basic Blue 3	20	0	30	B	1	37	B	B	659	42
39	Basic Blue 3	48	-4*	10	D	23*	46	B	-	650	42
40	Basic Blue 22	16	-17	-100	D	90	100	C	-	626	42
41	Basic Orange 22	8	-12	100	A	58	100	C	-	480	42
42	Basic Orange 40	51	41*	22	D	39*	48	C	-	440	42
43	Basic Red 22	6	20	95	A	22	85	A	A	534	10
44	Basic Red 46	77	74*	95	C	47*	98	C	C	530	42
45	Basic Violet 16	62	23	16	D	16	54	B	-	550	42
46	Direct Black 19	105	27	93	A	19	94	A	A	495	42
47	Direct Blue 10		-2	-17	D	13	-1	D	D	615	42
48	Direct Blue 14	99	84*	75	C	87*	86	C	C	596	48
49	Direct Blue 15	107	6	-7	D	13	25	D	D	605	41
50	Direct Blue 71	11	-27	27	D	65	100	C	-	564	42
51	Direct Blue 151	16	-6	44	B	44	100	C	-	540	42
52	Direct Brown 9		33	53	C	61	68	C	C	485	42
53	Direct Brown 106	31	0	-71	D	100	100	C	-	400	42
54	Direct Orange 46	28	14	-86	D	14	52	B	-	436	42
55	Direct Orange 60		20	49	B	26	55	B	B	420	42
56	Direct Red 7		23	20	D	16	50	B	-	550	42
57	Direct Red 7		23	20	D	16	50	B	-	550	42
58	Direct Red 23	26	12	54	B	28	92	A	-	550	42
59	Direct Red 81	108	34	20	D	33	0	D	D	560	41
60	Direct Red 254	70	12	56	B	16	28	D	-	512	42

0 1 0 4

Table 3 continued

Nr	dye stuff	DOC	DOC	DOC	DOC	col	col	col	elim	lambda	time
		start mg/l	ads %	elim %	cat	ads %	elim %	cat	cat	nm	d
61	Direct Yellow 27	3	-157	-267	D	14	44	B	-	436	42
62	Direct Yellow 44	104	22	70	A	0	73	A	A	392	48
63	Direct Yellow 132	100	20	-12	D	9	0	D	D	398	41
64	Direct Yellow 133	100	10	19	D	10	37	B	-	425	41
65	Dispers Yellow 3	8	100	50	C	0	0	D	-	425	48
66	Mordant Blue 13		9	5	D	23	83	A	-	560	40
67	Mordant Yellow 30	22	14	77	A	0	48	B	-	436	42
68	Pigment Yellow 151	62	87	95	C	87	96	C	C	399	10
69	Reactive Black 5	13	9	-53	D	9	50	B	-	560	28
70	Reactive Black 8	30	17	47	B	1	6	D	-	530	42
71	Reactive Blue 19	50	30	24	D	20	35	B	-	500	28
72	Reactive Blue 21	81	3	64	B	3	72	A	-	640	28
73	Reactive Blue 170	100	-4	-4	D	10	30	B	-	500	41
74	Reactive Brown 31	33	27	58	B	2	19	D	-	410	42
75	Reactive Green 12	106	17	27	D	22	47	B	-	655	42
76	Reactive Orange 16	20	15	15	D	15	37	B	-	490	28
77	Reactive Red 16	32	-6	-6	D	11	23	D	D	490	42
78	Reactive Red 24		16	23	D	4	-1	D	D	540	42
79	Reactive Red 35	16	25	69	A	24	42	B	-	498	30
80	Reactive Red 45	64	-10	-12	D	3	7	D	D	545	42
81	Reactive Red 133	10	20	20	D	24	53	B	-	530	30
82	Reactive Red 185	32	-6	-3	D	7	11	D	D	543	42
83	Reactive yellow 17	23	13	10	D	13	53	B	-	430	28
84	Reactive yellow 23	21	24	85	A	22	94	A	A	420	30
85	Reactive yellow 37	43	13	38	B	13	35	B	B	390	28
86	Reactive Yellow 42	22	18	-45	D	18	40	B	-	410	28
87	Reactive Yellow 155	32	22	47	B	1	3	D	-	450	42

* Adsorption equilibrium after 1 day

0105

Table 5 Results of the respirometric test

Nr	dye stuff	subst start mg/l	BOD end mg/g	subst COD mg/g	BOD/COD %	DOC elim %	col elim %	time d
2	Acid Black 2	100	0	1380	0	-	-	000000
		100	32		50	64	-	
		100	5		0	-	7	
		100	0		0	5	1	
8	Acid Blue 41	100	0	1335	0	-	-	000000
		100	15		10	0	-	
		100	40		0	-	6	
		100	340		30	12	10	
		250	350		60	-	-	
12	Acid Brown 159	100	0	714	0	-	-	000000
		100	45		0	51	-	
		100	0		0	-	10	
		100	510		71	14	9	
		500	320		45	-	-	
46	Direct Black 10	100	60	750	0	-	-	000000
		100	0		11	0	-	
		100	0		0	-	10	
		100	230		0	24	20	
		250	155		11	-	-	
62	Direct Yellow 44	100	45	1330	3	0	-	000000
		100	0		0	-	20	
		100	335		0	45	6	
		240	0		0	90	-	

abbreviations in table 3-5.

- DOC start = DOC concentration at the start of the test (mg/l)
 In the static test calculated from the stock solution,
 in the screening test measured value.
- DOC ads = DOC elimination after 3 hours = adsorption (%)
- DOC elim = DOC elimination at the end of the test (%)
- DOC cat = category of DOC elimination, according to table 1
- col ads = colour elimination after 3 hours = adsorption (%)
- col elim = colour elimination at the end of the test (%)
- col cat = category of colour elimination according to table 1
- elim cat = category of DOC and colour elimination, if both parameters show the same behaviour
- lambda = light absorption maximum of the dyestuff
 in the photometer (nm)
- time = duration of the test (days)
- adaptation = use of adapted inoculum in the test
- subst start = concentration of test substance at the start of the test,
 calculated from stock solution (mg/l)
- BOD end = measured biochemical oxygen demand at the end of test
 (mg/g)
- subst COD = chemical oxygen demand of the test substance (mg/g)
- BOD/COD = biodegradation of the test substance (%), calculated from
 BOD*100/COD

(Received in Germany 1 January 1986)

ETAD



ECOLOGICAL AND TOXICOLOGICAL ASSOCIATION
OF THE DYESTUFFS MANUFACTURING INDUSTRY
CH-4005 BASLE 5, SWITZERLAND

APPENDIX IV

REPORT CATEGORY: ONLY FOR USE WITHIN ETAD

REPORT NO.: E 3016-B

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R E P O R T E 3016-B

RESULTS FROM A STUDY ON EFFECTS
OF DYESTUFFS ON PLANT GROWTH
AND ON DYESTUFFS UPTAKE BY
PLANTS FROM SOIL

Approved by: TC

Issued by : ETAD Secretariat

Date: December 5, 1985

Date: February 28, 1986

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2

**ETAD PROJECT E3016:
Agricultural use of sludge contaminated
with colorants**

**Summary* of work carried out by ICI
during 1983 on the effects of three
dyestuffs on plant growth and the
analysis of the plants for possible
dyestuff contamination**

**Author: D Brown
ICI Brixham Laboratory
June 1984**

*** Note: The experimental detail of this
work has been given in separate
reports on the individual studies**



A recommendation made in the attached summary of Project E 3016, page 4, section 3.1, is that an investigation of the possible effect of a basic dyestuff on plant growth should be carried out. This work has now been completed using a basic dyestuff C.I. Basic Blue 26. This dye had no effect on the growth of three plant species up to and including the maximum dose of 1000 mg dyestuff per kg of dry soil.

If the necessity arises to continue this work there is no way to getting around the use of radio-labelled dyestuffs. Only by this means a quantitative balance of the fate of the test compounds and their metabolites will be possible.



1. Preface

The work reported was carried out in the context of the ETAD Project E 3016. The objective of this project has been formulated as follows:

Sludge recovered from biological effluent treatment plants is widely used for agricultural purposes. The objective of this project is to evaluate the persistence of the colorants in the soil and the possible uptake by the crop.

In a first phase the uptake from contaminated soil of two anthraquinone dyes, C.I. Acid Blue 25, C.I. No. 62055 and C.I. Disperse Blue 26, C.I. No. 63305, were studied. The reason for this selection was to include a water-soluble and a water-insoluble dye with best possible resistance to metabolic transformation. E.g. with azo dyes one would expect considerable losses of the parent compound by biological reductive cleavage of the azo group during the experiment. The study also included plant growth inhibition tests with three plant species.

In the case of azo dyes which are known to be metabolized in soil under anaerobic conditions forming aromatic amines, the question arises whether the metabolites formed are taken up by the plants. There is no doubt that the ideal way for such a study would be the use of radio-labelled materials. However, for cost reasons this pilot study was done with cold material. The model compound selected was the acid dye C.I. 13155. Upon reduction this compound generates 3,5-dichloro-aniline which was expected to be easily detected with a reasonable sensitivity in plant materials. This phase 2 study was carried out under similar conditions as the phase 1 tests.

Calculations based on rather conservative assumptions indicated that the level of any one dyestuff reaching agricultural land through contaminated sludge be of the order of 1 mg dyestuff per kg of surface soil (20 cm depth). The concentrations tested were 1, 10, 100 and 1000 mg of dyestuffs per kg of dry soil.

In the following a summary of the work is given. The experimental details are described in separate reports on the individual studies.

This pilot study indicates that the three dyestuffs investigated are not taken up from the soil in any significant amount. None of the dyestuffs had any observable effect on plant growth at the dose level likely to occur under practical conditions.

1 INTRODUCTION

During the treatment of aqueous effluents in sewage treatment plants many dyestuffs are removed by sorption processes onto sludge. Such sludge may in turn be used as a fertiliser for agricultural land and calculations based on "reasonable worst case" assumptions indicate that the level of any one dyestuff reaching agricultural land through this route may be of the order of 1 mg dyestuff/kg of land.

The work carried out by ICI in support of ETAD project E3016 was to grow 3 different species of plant in soils to which 2 anthraquinone and 1 azo dyestuff were separately added at levels up to 1 g/kg, both to assess any effects on growth, and to measure any uptake by the plants. In addition, with the azo dyestuff additional analysis for presence of 2,5-dichloroaniline, the probable anaerobic cleavage metabolite of the dye, was also undertaken.

2 SUMMARY

2.1 Dyestuffs tested

<u>Anthraquinone type:</u>	Acid Blue 25 and Disperse Blue 26 (work carried out Jan/Feb 1983)
<u>Azo type:</u>	Acid Dye CI No 13155 (work carried out Oct/Nov 1983)

2.2 Plant growth studies

The plants used were Sorghum wheat (Sorghum bicolor), Sunflower (Helianthus annuus) and Soya (Glycine max). The selection of the plant species and the general procedure used were based on the UK Health and Safety Commission Approved Code of Practice for the Determination of Ecotoxicity.

The test species were exposed to four concentrations of each dye, 1, 10, 100 and 1000 mg dyestuff/kg dry soil, and the test was started by sowing the plant seeds into the dosed soils and a control.

The test was carried out over a total period of 25 days, of which approximately 4 days was for germination/emergence and 21 days for growth. At the end of the test the plants were cropped at soil level and the foliage weighed and analysed.

2.3 Effects on germination and emergence

None of the dyestuffs tested had any significant effect on germination and emergence at any of the levels tested.

2.4 Effect on growth

None of the dyestuffs had any significant effect on plant growth up to, and including, the 100 mg/kg test level.

All three dyestuffs had a marked effect on the growth of the Sorghum and the Sunflower at the 1000 mg/kg test level, but only a marginal effect on the Soya.

Apart from the growth reduction, the plants exposed to the dyestuffs showed no other physical effect when compared with the controls.

2.5 Analysis for dyestuff

2.5.1 Plants

No dyestuff could be detected in the foliage of any of the plants grown in soil with levels of dyestuff up to 100 mg/kg.

At the 1000 mg/kg soil level the dyestuffs could just be detected in the foliage of all three plants, but levels were low (maximum 2 mg dyestuff/kg foliage).

2.5.2 Soil

Analysis of the soil for dyestuff after the growth test gave a variable recovery from 25 to 85%, with the higher recoveries at the higher exposure levels.

2.6 Analysis for 2,5-dichloroaniline (anaerobic cleavage product of CI No 13155)

Analysis of the dyestuff CI No 13155 showed that it contained approximately 1% of 2,5-dichloroaniline as an impurity. Analysis of the plants showed only trace amounts of the amine (max 0.2 mg/kg) at the 1000 mg dyestuff/kg soil exposure. Analysis of the soil at the 1000 mg/kg dyestuff level showed 2 mg/kg of 2,5-dichloroaniline or 20% of the level which would have been expected on the basis of its presence in the dyestuff as a 1% impurity.

3 CONCLUSIONS AND RECOMMENDATIONS

3.1 Effects on plant growth

The level at which all three dyestuffs show an effect on plant growth is three orders of magnitude higher than the calculation of a "reasonable worst case" level for contamination of agricultural land by a dyestuff in sewage sludge. For these particular dyestuffs this appears to be a satisfactory safety margin. Although it might be challenged on the basis of the possible additive effects of many dyestuffs over several years, this argument can be countered by our knowledge of the anaerobic degradation of dyestuffs. Also, if dyestuffs were actually present in soil at levels anywhere near those which might be considered critical, their presence would probably be visible and certainly amenable to analytical detection.

In regard to possible adverse effects of other dyes at such lower levels, since dyes have a variety of structures it is not justifiable to make any general statement based on the results with three materials. However, in the complete absence of any indication of a problem due to dyes in sewage sludge, and the apparent absence of any pressure from regulatory authorities, there seems to be no urgent case for further work in this area. A possible exception would be a study on a basic dye, which as a class are known to absorb strongly onto sewage sludge, and which also exhibit toxic properties to a number of other organisms.

3.2 Uptake by plants

Without using radio-labelled materials it is impossible to state with complete certainty that a substance or metabolite is not taken up. However, the work carried out provides a reasonable demonstration that with the three dyestuffs investigated, the intact dyestuff is not taken up into plant foliage in any significant amount. As above, it is not justifiable to extrapolate this to dyestuffs in general, but again there seems to be no urgent case for further work in this area.

With regard to the uptake of dye metabolites from soil, or the possible metabolism of dyes in plants following uptake, this work has not addressed these questions except for the possible metabolite, 2,5-dichloroaniline from the azo dyestuff CI 13155. As indicated above, for such work to be carried out with certainty, radio-labelled materials are necessary. The cost of such radio-label synthesis and the subsequent work does not seem justified at this present time.

4 TECHNICAL REPORTS ON THE WORK CARRIED OUT

ICI Brixham Laboratory:

BLS/A/0193 - The toxicity of two dyes Acid Blue 25 and Disperse Blue 26 to Sorghum (Sorghum bicolor), Sunflower (Helianthus annuus) and Soya (Glycine Max), March 1983

BL/A/2407 - The toxicity of dyestuff CI 13155 to Sorghum (Sorghum bicolor), Sunflower (Helianthus annuus) and Soya (Glycine Max), December 1983

ICI Organics Division, Research Department

NEW 5949/12 - ETAD Project 3016 (Analytical Report corresponding to BLS/A/0193). 4 March 1983

NEW 5949 - ETAD Project 3016 (Analytical Report corresponding to BL/A/2407) 24 November 1983

BLS/A/0193

3

THE TOXICITY OF TWO DYES AND PAGE 25 AND
DISPERSE BLUE 26 TO SORGHUM (Sorghum bicolor),
SUNFLOWER (Helianthus annuus), AND SOYA (Glycine max)

Authors: R D Stanley
J F Tapp

Issued by: L F Reynolds
March 1983

INTRODUCTION

Samples of two dyes, Acid Blue 25 and Disperse Blue 26 (Brixham test substance numbers J656 and J657 respectively), were submitted by Organics Division, Imperial Chemical Industries PLC, for a Growth Inhibition Test with Terrestrial Plants. The test method used is based on the HSC Approved Code of Practice for the determination of ecotoxicity.

SUMMARY

Acid Blue 25

Sorghum bicolor - EC₅₀* between 100 and 1000 mg/kg dry soil (estimated at about 520 mg/kg²).

Helianthus annuus - EC₅₀ value between 100 and 1000 mg/kg dry soil (estimated at about 290 mg/kg).

Glycine max - EC₅₀ value >1000 mg/kg dry soil.

Disperse Blue 26

Sorghum bicolor - EC₅₀ value between 100 and 1000 mg/kg dry soil (estimated at about 470 mg/kg).

Helianthus annuus - EC₅₀ value between 100 and 1000 mg/kg dry soil (estimated at about 670 mg/kg).

Glycine max - EC₅₀ >1000 mg/kg dry soil.

* EC₅₀ that concentration of chemical that will reduce the net weight growth of the plant by 50%.

† Estimated EC₅₀ values have been determined by applying computer PROBIT analysis to the available data.

Neither of the two dyes had any significant effect on the emergence of any of the three species at the tested concentrations.

Apart from the actual growth reduction the plants exposed to the dyes showed no other physical effect when compared with the controls.

MATERIALS AND METHOD

Three plant species were tested: Sorghum bicolor (sorghum), Helianthus annuus (sunflower), and Glycine max (soya). The test species were exposed to four concentrations of each dye, 1, 10, 100 and 1000 µg/kg dry soil for three weeks. Clean soil Controls were also run for each species in each study. The dye was introduced into the soil by mixing the dye with silver sand and then mixing this sand with the soil in the ratio 1 sand : 9 soil. The soil used was a commercially available potting compost with a low organic content, called "Special Mix".

For each tested concentration there were four replicate pots. Into each pot eight seeds were sown which were thinned to four plants per pot by removing the weakest plants one week after emergence.

At the end of the three-week test period the foliage from each pot was cropped at soil level and weighed (see Table 1).

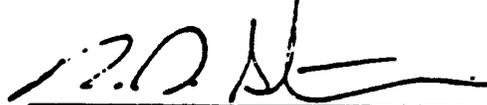
In addition to determining any growth inhibition, the cropped foliage was sent to Organics Division, Blackley, to determine whether there had been any dye uptake (these data are not presented in this report).

The test was carried out during the period 24.Jan.83 to 18.Feb.83.

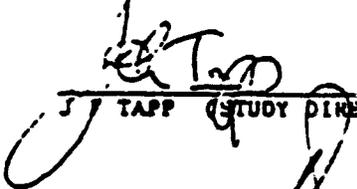
The study numbers were J656/A (Ail Blue 25) and J657/A (D. para Blue 26). All records and original data relevant to the growth inhibition studies are stored in the Brixham Archive.

RESULTS

Table 1 shows a summary of the results derived from the weights at the end of the test period. The results have been presented in the following way: the control weights for each species for each study have been summed and the average foliage weight per pot determined, the treated foliage weights are then shown as a percentage of the average control weight.


R D STANLEY (PRINCIPAL INVESTIGATOR)

4.3.83


J TAPP (STUDY DIRECTOR)

4-3-83


L F REYNOLDS

4.3.83

0118

TABLE 1

Shoot weights (per pot) shown as a percentage
of the average control weight (per pot)

<u>Acid Blue 25 (J656/A)</u>		Treatment Concentration (mg/kg)				Mean Control Weights
Species	Replicate	1	10	100	1000	
<u>SORGHUM</u>	1	96	88	81	45	
<u>BICOLOR</u>	2	83	96	87	26	
(SORGHUM)	3	93	91	78	16	
	4	95	101	70	46	
MEAN FOLIAGE WT/POT		3.7 g	3.9 g	3.3 g	1.4 g	4.1 g
<u>HELIANTHUS</u>	1	110	82	96	19	
<u>ANNUUS</u>	2	89	113	94	17	
(SUNFLOWER)	3	93	81	64	15	
	4	72	102	108	15	
MEAN FOLIAGE WT/POT		7.0 g	7.2 g	6.9 g	1.3 g	7.7 g
<u>GLYCINE</u>	1	80	103	99	91	
<u>MAX</u>	2	94	120	117	103	
(SOYA)	3	82	105	130	78	
	4	88	115	85	89	
MEAN FOLIAGE WT/POT		8.0 g	10.3 g	10.0 g	8.4 g	9.3 g
<u>Disperse Blue 26 (J657/A)</u>						
Species	Replicate					
<u>SORGHUM</u>	1	96	97	88	26	
<u>BICOLOR</u>	2	100	105	93	18	
(SORGHUM)	3	92	81	102	24	
	4	85	92	93	13	
MEAN FOLIAGE WT/POT		2.5 g	2.5 g	2.5 g	0.6 g	2.7 g
<u>HELIANTHUS</u>	1	86	110	108	18	
<u>ANNUUS</u>	2	98	136	136	27	
(SUNFLOWER)	3	132	130	138	25	
	4	132	127	114	42	
MEAN FOLIAGE WT/POT		5.5 g	6.2 g	6.1 g	1.4 g	5.0 g
<u>GLYCINE</u>	1	117	105	127	85	
<u>MAX</u>	2	99	105	101	63	
(SOYA)	3	101	94	110	75	
	4	89	94	96	73	
MEAN FOLIAGE WT/POT		9.8 g	9.6 g	10.5 g	7.2 g	9.7 g

IMPERIAL CHEMICAL INDUSTRIES PLC
ORGANICS DIVISION
DIVISION ANALYTICAL GROUP

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(4)

ETAD PROJECT E7016 : AGRICULTURAL USE OF SLUDGE CONTAMINATED
WITH COLOURANTS

4th March, 1983. (5949/12)

1. INTRODUCTION Plant growth inhibition tests for two anthraquinone dyes.

Acid Blue 25 and Disperse Blue 26 have been carried out on three different types of plant at varying dye/soil concentrations. After the 21 day test period had expired, an assessment of growth reduction was made by cropping the plants at soil level and analysing both plants and soil for dye content, using the extraction procedures described below.

The plant species used were Sorghum bicolor (grain sorghum), Helianthus annuus (sunflower) and Glycine max (soya), grown in soils containing 0, 1, 10, 100 and 1000mg dye/kg soil on a dry weight basis.

2. EXPERIMENTAL

2.1 ACID BLUE 25. Extraction procedures.

2.1.1. Plants

The plants were cut up into small pieces and blended for 2 minutes with 100mls of 1:1 acetone water. Suspended solids were filtered off, washed with acetone, the combined filtrates rotary evaporated down to low volume and diluted to 25mls with water. This solution contained some suspended green material which was filtered off and the filtrates were then examined for dye content by HPLC.

2.1.2. Soil

Samples of the soil were Soxhlet extracted overnight with water and the extracts examined by HPLC for dye content using the following conditions:-

Column : 10cm Spherisorbs 5 ODS
Solvent: 70:30 acetonitrile/H₂O containing 0.2% cetyl trimethyl ammonium bromide
Flow: 2mls/min.
Injection: 50 µl
Wavelength: 580 nm

2.2 DISPERSE BLUE 26. Extraction Procedures

2.2.1. Plants

The plants were processed in the same way as those for Acid Blue 25 above, except that they were blended with acetone alone and evaporated down to

about 4 ml and then adjusted to a final volume of 50ml's with acetone. Analysis of dye content was then carried out by HPLC using the following conditions.

Column: 25cm Hypersil ODS
 Solvent: 59:40:1 H₂O/THF/Acetic acid.
 Flow: 2mls/min
 Injection: 100 µl
 Wavelength: 580 nm

2.2.2. Soil

Samples of the soil were soaked in acetone and placed in a sonic bath for 15 minutes to fully extract the dye. The supernatant liquid was filtered off and the dye content measured by visible spectroscopy. A Perkin Elmer 330 UV/visible spectrophotometer was used, the dye wavelength monitored was 620nm and experimental extracts were assessed against dyestuff standards of appropriate concentration.

3. RESULTS

3.1 ACID BLUE 25

DYE/SOIL CONCENTRATION µg/kg dry weight basis	PLANT SPECIES			Dye content of soil(dry). After Growth Test mg/kg
	SORGHUM WHEAT	DYE CONTENT PPM SOYA	SUNFLOWER	
CONTROL (0)	N.D.< 0.2	ND< 0.1	ND < 0.05	N.D < 0.1
1	"	"	"	0.5
10	"	"	"	4.4
100	"	"	0.1	
			0.05	58
1000	0.2 < 0.2		0.3	
		0.5	2.0	884

3.2 DISPERSE BLUE 26

DYE/SOIL CONCENTRATION mg/kg.	PLANT SPECIES			DYE CONTENT OF DRIED SOIL AFTER GROWTH TEST mg/kg.
	SORGHUM WHEAT	SOYA	SUNFLOWER	
CONTROL (0)	N.D. < 1	N.D. < 0.6	N.D. < 0.6	N.D. < 2
1	"	"	"	NOT SAMPLED
10	"	"	"	2.7
100	"	"	"	28
1000	N.D. < 4	2	"	74.5

4. CONCLUSIONS

(a) 25-50% of the added dye is recovered from the soil after the growth test at lower dye concentrations, but this rises to 75-85% at higher dye concentrations.

(b) The dye up-take by the plants is very low, (usually none detected) with detections only upto 2ppm even from 1000ppm dosed soils.

(c) Typical detection levels of dye in plants and soil were 0.1-1ppm

(d) The apparent discrepancy between the detections limits for the different plant species is due to there being only a limited weight of plant available in some cases.

(e) If larger amounts of plant were available, it may be possible to achieve even lower detection limits than those quoted.

D.G. WILLIAMS.

NBW 5949/12.

Submitted to ETAD ASC Subcommittee meeting, 9-10th March, 1983
Basle. by A. Mathias.

APPENDIX

E3016 Initial ICI Results. Presented at ASC, Basel, 11th November, 1982.

1. ACID BLUE 26. (A water soluble dye).

1.1 "Soil" - specially prepared higher plant growth medium ex Brixham

186 ppm addition	92% recovery
24 ppm addition	60-70% recovery

1.2 Chinese Cabbage Leaves.

17 ppm addition.	100% recovery
------------------	---------------

1.3 Detection Limits

1.3.1. Soil Assuming a 20g sample with HPLC analysis, ~ 1 ppm

1.3.2. Chinese Cabbage Leaves 20g sample with HPLC and analysis, ~ 2ppm

2. DISPERSE BLUE 26 (A Water insoluble dye).

2.1 Soil:- as above

19ppm addition	89% recovery
----------------	--------------

2.2 Chinese Cabbage Leaves

19ppm addition	80% recovery
----------------	--------------

2.3 Detection Limits

2.3.1. Soil 20g sample, HPLC analysis, ~ 2ppm

2.3.2. Chinese Cabbage Leaves 20g sample, HPLC analysis, ~1ppm

5

BL/A/2407

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THE TOXICITY OF DYESTUFF CI 13155 TO SORGHUM
(Sorghum bicolor), SUNFLOWER (Helianthus annuus),
AND SOYA (Glycine max)

Authors R D Stanley
J F Tapp

Issued by B R H Williams
December 1983

BL/A/2407

THE TOXICITY OF DYESTUFF CI 13155 TO SORGHUM (Sorghum bicolor)
SUNFLOWER (Helianthus annuus), AND SOYA (Glycine max)

AUTHENTICATION

We, the undersigned, hereby declare that this study was performed under our supervision according to the procedures herein described, and this report represents a true and accurate record of the results obtained.

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29-11-83
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:  30.11.83

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BL/A/2407

THE TOXICITY OF DYESTUFF CI 13155 TO SORGHUM (Sorghum bicolor),
SUNFLOWER (Helianthus annuus), AND SOYA (Glycine max)

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4

1 INTRODUCTION

A sample of a dyestuff, CI 13155 (Brixham Test Substance Number K391), was submitted by Imperial Chemical Industries PLC, Organics Division, for a growth inhibition test on terrestrial plants. The test method used was the Guideline for Growth Inhibition in Terrestrial Plants from the HSE Approved Code of Practice.

Upon completion of the test the cropped foliage was despatched to Organics Division, Blackley for determination of dye uptake. These results will be reported separately by Blackley.

The study number was K391/A and the test was carried out between 10 October and 4 November 1983. All records and original data relevant to this study are stored in the Brixham Laboratory Archive.

2 SUMMARY AND CONCLUSIONS

The EC50* values for the 3 species of plant tested were all greater than 1,000 mg of dyestuff/kg of soil (dry weight).

All three species showed some growth reduction at the highest concentration (1,000 mg/kg), the plants grown in the lower concentrations were unaffected. There was insufficient growth reduction to calculate EC50 values.

Apart from the actual growth reduction the plants showed no other physical effect from exposure to the dyestuff.

The dyestuff had no significant effect on the emergence of any of the three species at the tested concentrations.

* EC50 - that concentration of chemical that will reduce the foliage weight of the plant by 50%

3 TEST SPECIES, GROWTH MEDIUM, AND TEST CHEMICALS

3.1 Test species

The three species used were:

- (a) Sorghum (Sorghum bicolor)
- (b) Sunflower (Helianthus annuus)
- (c) Soya (Glycine max)

The seeds were supplied by the Plant Production Dept, Jealott's Hill Research Station.

3.2 Growth medium

The growth medium used was a commercially available potting compost - Special Mix. For details of physical and chemical composition see Table 1.

3.3 Test chemical

The test chemical was a dyestuff supplied by Imperial Chemical Industries PLC, Organics Division, Blackley. The dyestuff was designated CI 13135 and the Brixham Test Substance Number was K391.

4 METHOD AND CONDITIONS

- 4.1 The HSE Method for Growth Inhibition in Terrestrial Plants that was used for this test is described in detail in the appendix to this report. The following changes or additions, which are included in SOP TX7, were made to the test.
- 4.1.1 No solvents were used to dissolve the dyestuff, instead the dyestuff was mixed with a small amount of sand in a mortar and pestle. This ground-up mixture was then shaken with the rest of the sand in a polythene bag before being incorporated into the soil by shaking in a polythene sack.
- The four tested concentrations were 1, 10, 100 and 1,000 mg of dyestuff/kg of soil (soil weight quoted on a dry weight basis).
- 4.1.2 The plant pots used were 10 cm diameter.
- 4.1.3 The seeds were sown in the treated soil on day minus 4 of the test. This meant that 50% emergence would occur during day 0 for all three species (Stanley and Tapp, 1982, Brixham Laboratory Report BL/A/2197). Assessments of growth were made on days 0, 7 and 21.
- 4.1.4 The plants were kept watered by a capillary matting sub-irrigation system. In addition the pots were examined twice daily and additional water supplied if necessary.
- 4.1.5 Eight seeds were sown in each pot and the resultant plants were thinned to four per pot on day 7.
- 4.1.6 The test was conducted in a glasshouse maintained at a nominal 20°C. A record was kept of temperature and humidity by means of max/min thermometer (see Table 2) and a thermohygrograph trace. Additional light was supplied during periods of poor natural daylight by means of mercury vapour lamps to provide a minimum day length of 14 hours.
- 4.1.7 No additional nutrients were supplied to the plants.

5

RESULTS

On day 21 the foliage was cropped at soil level and weighed (all 4 shoots from each pot were weighed together). These results can be found in Table 3.

When compared with the controls, plants of all species grown in concentrations 1, 10 and 100 mg/kg showed no significant growth reduction. All three species had significant growth reduction at 1,000 mg/kg; however this growth reduction was not sufficient to calculate an EC50 value.

None of the tested concentrations had a significant effect on emergence. On day 7, when the plants were thinned, all the pots were left with four healthy plants.

ACKNOWLEDGMENTS

This work was carried out in the glasshouses of the Plant Production Department, Weed Science Section, Jealott's Hill Research Station, Plant Protection Division. The authors would like to thank the staff of that section for their help and co-operation during the execution of this test.

TABLE 1

PHYSICAL AND CHEMICAL COMPOSITION OF SPECIAL MIX

A Particle size analysis

	mm	%
Pebbles	64 - 4	8.6
Granules	4 - 2	14.3
Very coarse sand	2 - 1	9.8
Coarse sand	1 - 0.5	11.6
Medium sand	0.5 - 0.25	17.9
Fine sand	0.25 - 0.125	17.3
Very fine sand	0.125 - 0.0625	9.1
Silt	0.0625 - 0.02	4.7
Clay	<0.02 mm	6.7

B Organic matter

3%

(determined by weight loss on ignition of dry soil)

C pH

5.75

D Wet:dry ratio

1.1:1

TABLE 2

RECORD OF MINIMUM AND MAXIMUM GREENHOUSE TEMPERATURES (°C)

Date	9.00 am		4.30 pm	
	min	max	min	max
OCT 10	12	24	19	26
11	17	25	25	30
12	16	22	21	25
13	18	22	20	25
14	15	21	22	32
*				
17	16	30	23	28
18	17	29	21	26
19	16	25	22	30
20	16	25	23	30
21	15	25	22	30
*				
24	15	30	22	25
25	17	30	21	28
26	15	23	22	25
27	16	24	20	28
28	13	26	20	29
*				
31	15	29	21	24
NOV 1	16	22	22	24
2	16	23	21	24
3	16	25	21	26
4	16	23	23	24
MEAN	15.7	25.2	21.6	27.1
VALUES	20.4		24.3	

22.4

* Temperatures not recorded at weekends

0-1-3-4

TABLE 3

RECORD OF TOTAL FRESH WEIGHT OF PLANT SHOOTS
PER POT AFTER 21 DAYS

(Each figure represents total weight per pot)

SPECIES	TREATMENT CONCENTRATION (mg/kg)	R E P L I C A T E				MEAN WEIGHT
		1	2	3	4	
<u>Glycine</u> <u>max</u> (Soya)	Control	10.49	12.20	11.99	10.85	11.38
	1	11.52	10.36	12.53	10.29	11.18
	10	9.91	13.95	13.44	10.34	11.91
	100	13.12	13.07	12.57	12.74	12.89
	1000	10.24	9.16	10.69	9.26	9.84
<u>Sorghum</u> <u>bicolor</u> (Sorghum)	Control	4.59	4.18	4.31	3.62	4.18
	1	2.97	4.36	4.08	3.98	3.85
	10	3.36	3.26	3.72	3.84	3.55
	100	3.90	4.30	4.47	3.52	4.05
	1000	2.70	2.61	2.62	2.65	2.65
<u>Helianthus</u> <u>annuus</u> (Sunflower)	Control	22.26	21.19	17.18	18.60	19.81
	1	21.57	15.60	14.59	19.01	17.69
	10	19.61	23.87	19.56	22.45	21.37
	100	18.50	19.49	18.05	28.49	21.13
	1000	15.03	11.52	11.97	12.72	12.81

GROWTH INHIBITION IN TERRESTRIAL PLANTS

- 1 **The test organism(s)**
The method described is intended to assess the influence of a contaminated soil on seed germination and early growth of higher plants. The test contains considerable procedural latitude which should make it relatively easy to operate.
- 2 **Three species are used:**
 - (a) Avena sativa (oats) or Sorghum bicolor (grain sorghum)
 - (b) Brassica napus (rape) or Helianthus annuus (sunflower)
 - (c) Medicago sativa (lucerne, alfalfa) or Glycine max (soya)
- 3 **Testing requirements**
The following test pattern should be used:
 - (a) A widely available agricultural soil sieved (0.5 cm) to remove coarse debris and with a total carbon content of not more than 1% and 10-20% clay minerals (vermiculite and peat based composts are not acceptable because of their high absorptive capacity).
 - (b) The chemical to be tested should be dissolved in water or in a volatile organic solvent and mixed with quartz sand at four different concentrations and a blank.
 - (c) The solvent is evaporated and the sand mixed into soil in the ratio 1 sand:9 soil.
 - (d) Ungerminated seeds should be sown in treated sand-soil mixture in 7-9 cm plastic pots within 24 h of adding the chemical to the sand, and the water content adjusted to suit the species.
 - (e) Temperature, humidity and light conditions should be suitable for maintaining "normal" growth of each species for a period of at least six weeks after emergence.
 - (f) Additional nutrients, where required, may only be added after the two week post emergence assessment.
- 4 **Observing and reporting of results for terrestrial plants**
The following should be recorded:
 - (a) Dates of emergence throughout the test.
 - (b) Main assessments are made at weekly intervals starting from the day in which 50% of the control seedlings have emerged.
 - (c) At 1 week after emergence plants are assessed and then thinned by removing the weakest seedlings.
- 5 **Assessment of terrestrial phytotoxicity**
Measurement of phytotoxicity is based on a visual assessment of the plants on a 0-3 scale:
 - 0 - no damage,
 - 1 - up to 20% damage,
 - 2 - 20-50% damage,
 - 3 - greater than 50% damage.
 - (a) Damage in any respect of plant growth which distinguishes it from the control, eg stunting, chlorosis, necrosis.
 - (b) Plants scoring 0 or 3 require no further analysis, but some quantitative measurements such as height or fresh weight should support the final visual assessments of 1 and 2.

(HSE Approved Code of Practice: Methods for the Determination of Ecotoxicity - Notification of New Substances Regulations 1982)

0 1 3 6

Appendix 2

A TEST PROCEDURE TO DETERMINE THE GROWTH INHIBITION OF A CHEMICAL TO TERRESTRIAL PLANTS

This procedure has been designed to meet the requirements of the HSE Notification of New Substances Regulations 1982 - Growth Inhibition in Terrestrial Plants.

1 OBJECTIVE

To assess the influence of a contaminated soil on seed germination and early growth of higher plants.

2 TEST MATERIAL

A sufficient quantity of the test material will be supplied by the sponsor. Information regarding handling procedures, hazardous properties and storage conditions will also be supplied by the sponsor.

3 TEST SPECIES

3.1 Species/Supplier

Sorghum bicolor (grain/crop sorghum), Helianthus annuus (sunflower), Glycine max (soya). The seed will be obtained from a reputable supplier.

3.2 Specification

The seed viability should be in excess of 90%.

3.3 Justification

The test species are readily available, easily grown, and are included in the HSE guidelines.

4 PLANT POTS

The plant pots will be new 10 cm diameter plastic pots and will be disposed of at the end of the test.

5 IDENTIFICATION OF TEST SYSTEM

Each pot will be labelled in waterproof ink with the following information :

- (i) Test species (initials of latin name ie SB, HA or GM)
- (ii) Study number
- (iii) Concentration in mg/kg
- (iv) Replicate number

AFAABD

0 1 3 7

6 GROWTH MEDIUM

The growth medium used will be commercially available soil based potting compost - 'Special Mix' - specifically designed for greenhouse use.

7 TEST CONCENTRATIONS

The concentrations tested will be 1, 10, 100 and 1000 mg of test material per kg of soil (dry weight basis) unless otherwise specified by the sponsor. There will be clean soil controls for each species for each test. Four replicates of each concentration will be set up.

8 PREPARATION OF GROWTH MEDIUM

The test material will be mixed with sand and this mixture will be incorporated into the Special Mix in the ratio 1:9.

The sand will be silver sand that has been washed, sieved through 0.5 mm, and oven dried.

The sand/test material mixture will be prepared by adding the test material to a small amount of the sand in a mortar and pestle and grinding the two together until finely and evenly dispersed. This will then be added to the remaining sand in a polythene bag and shaken for at least half a minute.

The final sand/test material mixture will then be added to an appropriate amount of Special Mix and shaken in a polythene sack for at least half a minute. Controls will be prepared with clean sand. (Note: for calculating amounts of soil needed one plant pot holds approximately 750 g of Special Mix as supplied).

NB. The test concentrations are quoted in kg of dry soil, therefore when weighing out the soil account must be taken of its moisture content. On the basis of previous work the wet/dry ratio of Special Mix as supplied will be taken as 1.1 : 1 for the purposes of this test method.

9 TEST CONDITIONS

9.1 Temperature

The test will be conducted in a glasshouse at a nominal 20°C, however, due to the nature of glasshouses wide variations from the nominal temperature will sometimes be experienced during periods of extreme weather conditions.

A daily record of maximum/minimum temperatures will be maintained from Mondays to Fridays and a thermohygrograph will be employed throughout the test.

AFAABD

9.2 Water

Water will be supplied by a system of capillary matting sub-irrigation. In addition the pots will be examined twice daily and further water supplied if necessary by 'top-down' watering.

9.3 Light

During periods of poor natural daylight, additional light will be supplied by mercury vapour lamps to provide a minimum day length of 14 hours.

9.4 Growth Medium

A representative sample of Special Mix will be taken and analysed for the following :

- (i) Particle size (dry sieving method)
- (ii) Organic matter (loss on ignition)
- (iii) pH
- (iv) wet/dry ratio

10 ANALYSIS

There will be no chemical analysis of the treated soil or the plant foliage unless otherwise requested by the sponsor.

11 PROCEDURE

Day minus 4

The treated soil will be prepared and put into the pots. Eight seeds will be sown into each pot of soil and the prepared pots will be placed on a bench in a suitable glass house.

Day 0

An assessment of emergence will be made.

Day 7

An assessment will be made of emergence and any growth effects seen will be noted. The plants will then be thinned to four per pot by removing the weakest plants.

Day 21

The phytotoxicity of the test material will be assessed. Any test where a visual assessment reveals no significant growth reduction of the plants grown in the treated soil requires no quantitative measurement of growth.

Any test where there is visual evidence of significant growth

AFAABD

0 1 3 9

BL/A/2407

THE TOXICITY OF DYE STUFF CI 13155 TO SORGHUM (Sorghum bicolor)
SUNFLOWER (Helianthus annuus), AND SOYA (Glycine max)

CIRCULATION

Copy
number

1	W Sharples	Organics Division, ICI PLC
2	D Williams	
3	B R H Williams	Brixham Laboratory. ICI PLC
4-6	D Brown	" " " "
7	J F Tapp	" " " "
8	R D Stanley	" " " "
9-11	Library	" " " "

TABLE 2

DYE/SOIL CONCENTRATION mg/kg DRY WEIGHT BASIS	SORGHUM WHEAT		PLANT SPECIES SOYA		SUNFLOWER		SOIL (dry) DYE CONTENT AFTER GROWTH TEST	
	DYE PPM	AMINE PPM	DYE PPM	AMINE PPM	DYE PPM	AMINE PPM	DYE PPM	AMINE PPM
CONTROL (0)	N.D. <0.1	N.D. <0.1	N.D. <0.1	N.D. <0.1	N.D. <0.1	N.D. <0.1	N.D. <0.05	N.D. <0.05
1	N.D. <0.1	N.D. <0.1	N.D. <0.1	N.D. <0.1	N.D. <0.1	N.D. <0.1	0.5, 0.8, 0.6	N.D. <0.05
10	N.D. <0.1	N.D. <0.1	N.D. <0.1	N.D. <0.1	N.D. <0.1	N.D. <0.1	5.6, 8.5, 6.5, 5.9	0.1, 0.1, 0.1
100	N.D. <0.1	N.D. <0.1	N.D. <0.1	N.D. <0.1	APPROX 0.1	N.D. <0.1	61.2, 58.4	0.3, 0.3
1000	APPROX 0.1	APPROX 0.2	0.2	APPROX 0.1	0.5	673, 738, 823, 749	2.1, 2.2, 1.8, 2.2	

N.D. = None detected

Dye = Acid dye C.I. 13155

Amine = 2,5-dichloroaniline

INTERNATIONAL CHEMICAL INDUSTRIES PLC
ORGANICS DIVISION

DIVISION ANALYTICAL GROUP

WORKING DOCUMENT FOR USE WITHIN THE ASC/ESC COMIT

ETAD PROJECT E3016: AGRICULTURAL USE OF SLUDGE CONTAMINATED WITH COLOURANTS

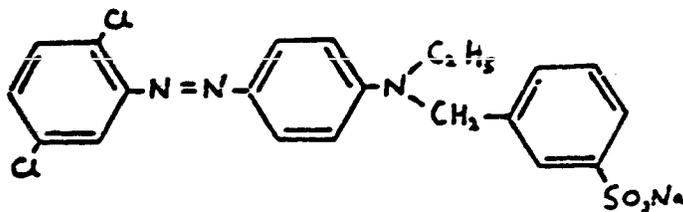
24th November 1983

NEW 5949

1. INTRODUCTION

As agreed at the 27th ASC meeting, Acid dye C.I. No. 13155, synthesised by Sandoz, was used in a plant growth inhibition study with three plant species at varying dye/soil concentrations.

Previous work carried out on two *anthraquinone* Disperse Dyes (see report dated 4th March 1983) involved the analysis of both plant and soil for dye only. In this study analyses for both Acid dye C.I. 13155 and the possible metabolite 2,5 dichloroaniline were undertaken.



ACID DYE C.I. 13155

A preliminary examination of the dye, as received, by both gas chromatography and liquid chromatography showed it to contain 1.0% 2,5 dichloroaniline.

The plant species used in the study were Sorghum bicolor (grain sorghum), Helianthus annuus (sunflower) and Glycine max (Soya), grown in soils containing 0, 1, 10, 100 and 1000 mg dye/kg soil on a dry weight basis.

2. EXPERIMENTAL

2.1 Recovery Experiments

Wherever possible recovery experiments were carried out with the individual plant species and soil (for results see Table 1).

2.2 Extraction Procedures

2.2.1 Plants

The plants (20 g/test) were cut up into small pieces and blended with 80 ml of 1:1 acetone:N/10 hydrochloric acid for 3 minutes, filtered through glass fibre paper and the filtrate diluted to 100 ml with 1:1 acetone:N/10 hydrochloric acid. This solution was then examined by LC using the conditions described below.

2.2.2 Soil

Samples of the soil (20 g/test) were soaked in 50 ml of 1:1 acetone:N/10 hydrochloric acid and placed in a sonic bath for 15 minutes. After being allowed to stand for 30 minutes the supernatant liquor was examined by LC (for results see Table 2) using the following conditions.

Column : 25 cm Hypersil ODS

Eluent : 50/49/1 Acetonitrile/0.2 M Ammonium acetate/Acetic acid

Flow : 2 ml/min

Injection : 200 μ l

Wavelength : 450 nm dye, 245 nm (using 295 nm as a reference wavelength) for the amine

3. RESULTS

Table 1

Recoveries from Soil and Plants

Sample	ppm dye added	ppm amine added	% Recovery
Soil	0.14	0.15, 0.15	82, 84 87
Soil	101, 108		70, 69
Sunflower	0.13	0.13	105 100
Soya	0.13	0.14	68 110

4. CONCLUSIONS

- (a) Only 60-75% of the added dye was recovered from the soil after the growth tests.
- (b) Only very low levels of the dye and 2,5 dichloroaniline, were detected in plants exposed to the higher dye concentrations. The amine detected in soil can be attributed to the residual 1.0% 2,5 dichloroaniline present in the dye.

D. Williams

D.C. Williams

6

IMPERIAL CHEMICAL INDUSTRIES PLC
BRIXHAM LABORATORY

Project E3016. Agricultural Use of Sludge Contaminated with Colourants

September 1985

BLS/A/0297

CI BASIC BLUE 26: Toxicity to Sorghum (Sorghum bicolor)
Sunflower (Helianthus annuus) and soya (Glycine max)

Authors A J Windeatt
 J F Tapp

Approved by L F Reynolds
 September 1985

INTRODUCTION

A sample of the dyestuff Basic Blue 26 (Brixham Laboratory Test Substance Number N055) was examined in a Growth Inhibition Test with Terrestrial Plants. The test method used is based on the HSC Approved Code of Practice for the determination of ecotoxicity.

SUMMARY

Sorghum bicolor - No effect on growth up to the maximum test level of 1000 mg/kg

Helianthus annuus - No effect on growth up to the maximum test level of 1000 mg/kg

Glycine max - No effect on growth up to the maximum test level of 1000 mg/kg

The dye also had no significant effect on the emergence of any of the three species at the tested concentrations.

None of the plants exposed to the dye showed any physical effect when compared with the controls.

MATERIALS AND METHODS

Three plant species were tested: Sorghum bicolor (sorghum), Helianthus annuus (sunflower) and Glycine max (soya). The test species were exposed to four concentrations of the dye, 1, 10, 100 and 1000 mg/kg dry soil for three weeks. Clean soil controls were also run for each species in each study. The dye was introduced into the soil by mixing the dye with silver sand and then mixing this sand with the soil in the ratio 1 sand : 9 soil. The soil used was a commercially available potting compost with a low organic content, called "Special Mix".

For each tested concentration there were four replicate pots. Into each pot eight seeds were sown which were thinned to four plants per pot by removing the weakest plants one week after emergence.

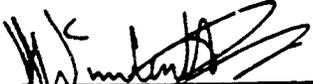
At the end of the three-week test period the foliage from each pot was cropped at soil level and weighed (see Table 1).

The test was carried out during the period 14 February - 11 March 1985.

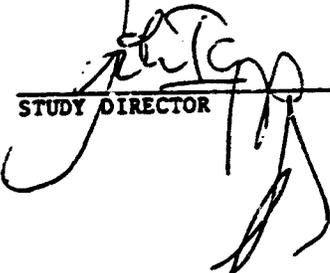
The study number was N033/A. All records and original data relevant to the study are stored in the Brixham Laboratory Archive.

RESULTS

Table 1 shows a summary of the results derived from the weights at the end of the test period.



PRINCIPAL INVESTIGATOR A J Windeatt 10-9-85



STUDY DIRECTOR J F Tapp 10-9-85



PROJECT MANAGER L F Reynolds 10/9/85

TABLE 1

STUDY NO N033/A SHOOT WEIGHTS (g) (PER POT)

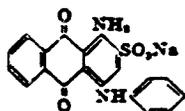
Species	Replicate	Control	Treatment concentration (mg/kg)			
			1	10	100	1000
SORGHUM	1	0.33	0.40	0.45	0.58	0.41
BICOLOR	2	0.28	0.46	0.50	0.54	0.47
(Sorghum)	3	0.37	0.40	0.43	0.45	0.50
	4	0.31	0.36	0.42	0.41	0.51
MEAN FOLIAGE WT/POT		0.32	0.41	0.45	0.50	0.47
HELIANTHUS	1	1.42	1.37	1.59	1.79	1.36
ANNUUS	2	1.19	1.39	1.56	1.21	1.39
(Sunflower)	3	1.41	1.45	1.09	1.69	1.48
	4	1.34	1.78	1.52	1.53	1.19
MEAN FOLIAGE WT/POT		1.34	1.50	1.44	1.56	1.36
GLYCINE MAX	1	3.95	3.53	4.32	4.95	3.30
(Soya)	2	3.88	4.56	4.82	4.31	4.51
	3	4.43	4.32	4.48	4.87	4.18
	4	4.13	4.16	4.20	4.55	5.44
MEAN FOLIAGE WT/POT		4.10	4.14	4.46	4.67	3.86

ETAD



7. Dyestuffs tested in Project E 3016

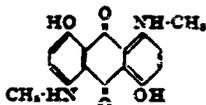
1. C.I. Acid Blue 25, C.I. No. 62055



Purity Specifications:

Active : 82%
Dispersing Agent: 12%
Inorganics : 1%
Water : 5%

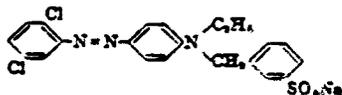
2. C.I. Disperse Blue 26, C.I. No. 63305



Purity Specifications:

Active : 26%
Dispersing Agent: 68%
Wetting Agent : 2%
Water : 4%

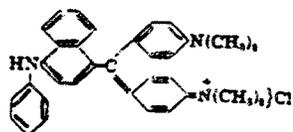
3. Acid Dye C.I. No. 13155



Purity Specifications:

HPLC pure one single peak
Active: 95%
NaCl : 5%

4. C.I. Basic Blue 26, C.I. No. 44045



Purity Specifications:

Active : 93%
Dispersing Agent: 6%
Dedusting Agent : 1%