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Detection of Carcinogenic Amines from Dyestuffs or Dyed Substrates

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ABSTRACT

The second amendment to 'German Regulations on Consumer Goods' states that azo dyestuffs, which can release one or more of the listed 20 carcinogenic amines, should no longer be used in dyeing consumer goods. Many acid and direct dyes which liberate harmful amines such as benzidine, o-*tolidine and* o-*dianisidine after reduction are, however, still used. In this study, it was surprising to find that some dyestuffs or dyed substrates released carcinogenic amines such as 4-aminodiphenyl, 2-naphthylamine, 2,4-toluenediamine and 4,4'-diaminodiphenylmethane, although such amines had not been employed as intermediates in the manufacture of the dyestuffs. Benzidine was also detected from a dyestuff which was not made from benzidine. The 2-naphthylamine residues were sourced as being due to the use of 1-naphthylamine contaminated with 2-naphthylamine. 4-Aminodiphenyl was formed by dediazoniation and a subsequent coupling reaction between the benzenediazonium ion and aniline. Benzidine was derived from dediazoniation and a subsequent self-coupling reaction of the diazonium ion of 4-nitroaniline. 2,4-Toluenediamine and 4,4'-diaminodiphenylmethane arose from the alkaline hydrolysis of the readily accessible moiety of the corresponding base units in PU foams or PU finishing agents.* © *1997 Elsevier Science Ltd. All rights reserved*

Keywords: ECO labels, carcinogen, diazotization, dediazonization, azo, dyestuff.

INTRODUCTION

Western Europe, especially Germany, endows regularly used goods including textiles and leathers with ECO labels to guarantee consumers that purchased

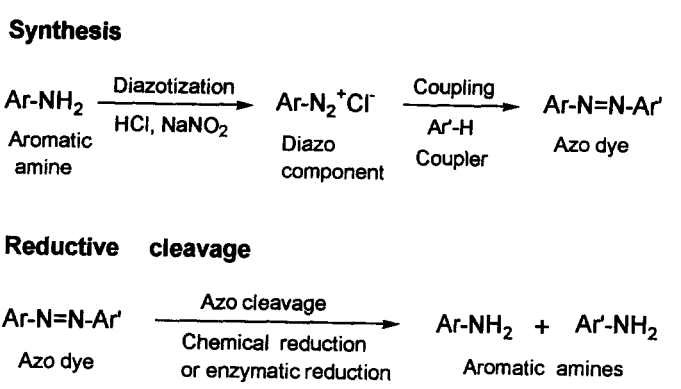
TABLE 1
Twenty Carcinogenic Amines

2-Aminoazotoluene	4-Aminodiphenyl
2-Amino-4-nitrotoluene	Benzidine
4-Chloroaniline	4-Chloro-2-toluidine
4-Cresidine	2,4-Diaminoanisole
4,4-Diaminodiphenylmethane	*o*-Dianisidine
3,3′-Dichlorobenzidine	3,3′-Dimethylbenzidine
3,3′-Dimethyl-4,4′-diaminodiphenylmethane	4,4′-Methylene-bis-(2-chloroaniline)
2-Naphthylamine	4,4′-Oxydianiline
4,4′-Thiodianiline	2,4-Toluenediamine
o-Tolidine	2,4,5-Trimethylaniline

products are free from environmental pollution hazards, and are not toxic. ECO labels are based on the standards and limited values established by various associations, research institutes and consulting companies; these are typified by the eco-tex®, GuT®, Öko-Tex Standard 100, TOX PROOF® and EU environmental symbols among representative ECO labels. In addition, many competitive ECO labels have been introduced especially in Germany. Among ECO labels, the 'Öko-Tex Standard 100', which is given by 'International Association for Research and Testing in the Field of Textile Ecology', including 12 research institutes in individual countries, is one of the best known and most widely used.

It is well established that many aromatic amines such as benzidine and 2-naphthylamine are carcinogens.[1–5] Only relatively recently have regulations been introduced to prohibit the use of these harmful amines as intermediates for the manufacture of dyestuffs. ECO labels thus form part of a necessary control system, and additionally the German government has declared a second amendment to 'German Regulations on Consumer Goods' (Bedarfsgegenstandverordnung, amended on 15 July 1994), that any azo dye, which could release one or more of the listed 20 carcinogenic aromatic amines (see Table 1), should not be used in the manufacture of consumer goods. The import of consumer goods dyed with azo dyestuffs containing the harmful amines was prohibited from 1 January 1995 and sales of already imported goods from 1 July 1995. However, the import prohibition date was postponed until 31 March 1996 and the circulation prohibition date to 30 September 1996 in the fourth amendment (20 July 1995).

As shown in Scheme 1, azo dyestuffs which are obtained by coupling the diazonium ion of aromatic amine with an aromatic coupling component will release aromatic amines under reductive cleavage conditions. Conditions that can cause reductive splitting of azo dyes can be found, for example, in a reductive chemical medium (chemical reduction), and in the human organism as a result of the action of intestinal bacteria or azo reductase in the liver



Scheme 1

or other organs on any azo dyestuff in the body (enzymatic reduction). Toxicologically relevant azo dyestuffs are those whose reductive splitting can lead to the release of carcinogenic aromatic amines.

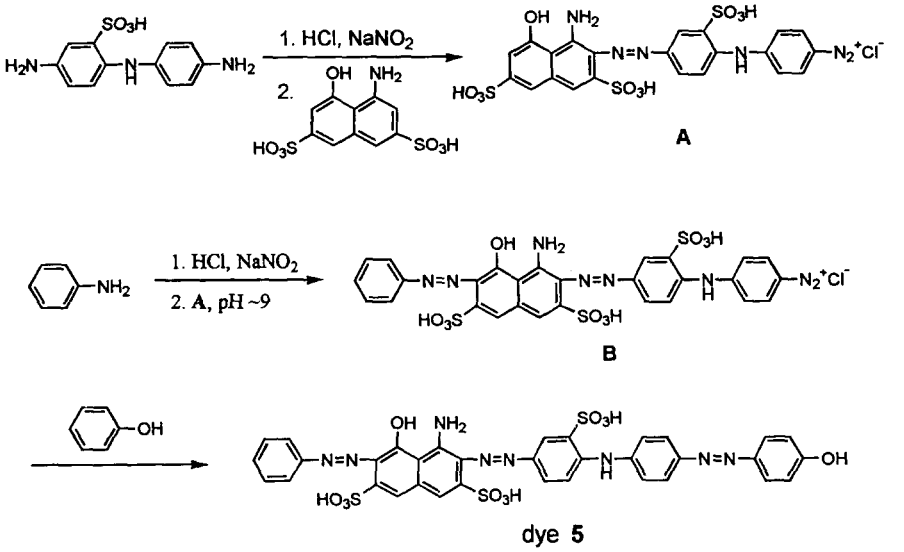
We have now established an experimental method to identify whether dyestuffs and dyes extracted from dyed substrates contain such harmful amines or not. Whilst there are some acid and direct dyes which still release carcinogenic amines such as benzidine, *o*-tolidine and *o*-dianisidine after reduction, it was surprisingly found that 4-aminodiphenyl, 2-naphthylamine, 2,4-toluenediamine and 4,4′-diaminodiphenylmethane were detected in many cases, although these amines had not been used in the dye synthesis. Benzidine was also detected from a dyestuff that did not use benzidine as an intermediate for its manufacture. In this paper the sources and the reasons for these results are described and discussed.

EXPERIMENTAL

Materials

The standard samples of the 20 carcinogenic aromatic amines were purchased from Aldrich, Sigma, Tokyo Kasei and Fluka. All dyes and intermediates described in this paper were furnished by various dyestuff companies.

A large number of dyes and consumer goods were investigated. The investigation was primarily concerned with establishing a viable method for the detection and characterisation of carcinogenic arylamines. It was not concerned with specifically identified dyes. The dyes **1**–**4** were of unknown total structure, and illustrate cases where specific amines were isolated and possible sources of them evaluated. Dye **5** is shown in Scheme 2.



Scheme 2

The exact amounts of the detected amines were not measured, the method reported being used only to establish whether the amount was above the maximum allowable level by comparisons with the pertinent known weight of an authentic sample.

General test method

Commercial dyestuffs were reduced directly with sodium dithionite in aqueous alkaline solution. Colored substrates were extracted and then the extracts were cleaved reductively with sodium dithionite in aq. alkaline solution. The reduced solution was extracted with ethyl acetate, the extracts separated on TLC and the components identified and reconfirmed by GC/MS (Varian 3400, EI mode). All extracted amines were confirmed by comparison with authentic samples. The dyestuffs and dyed substrates discussed below were found to contain carcinogenic amines above the allowed limit value (50 mg/kg for dyestuff and 5 mg/kg for dyed substrate).

RESULTS AND DISCUSSION

The azo dyes were cleaved reductively with sodium dithionite in aq. alkaline solution to ascertain whether they released any carcinogenic aromatic amines or not. The amines were identified using TLC, GC or LC by comparison with standard reference samples. However, these methods are often uncertain,

because some compounds have the same R_f values on TLC and/or the same retention time on GC and/or LC. For more positive confirmation, GC/MS was necessary.

In the main, structural isomers of an aromatic amine have different retention times on GC and/or different fragmentation patterns in MS. For instance, although the carcinogenic amine 2,4-diaminoanisole and dye-intermediate 2,5-diaminoanisole have the same retention time on GC, their mass fragmentation patterns are significantly different (Fig. 1). A harmful amine 4-aminodiphenyl and its structural isomer 2-aminodiphenyl could be readily differentiated since they are separated on GC and give different mass spectra (Fig. 2). However, 2-naphthylamine has the same mass spectrum and a very similar retention time as 1-naphthylamine (Fig. 3), and firm identification of 2-naphthylamine always requires comparison with a standard reference sample in the gas chromatogram.

2-Naphthylamine

Whilst 2-naphthylamine is not employed in the synthesis of azo dyes, 1-naphthylamine is a useful intermediate in some azo dyes. However, 2-naphthylamine was almost always detected whenever the reductive cleavages of some dyestuffs liberated 1-naphthylamine. 2-Naphthylamine is obtained as a side product in the manufacture of 1-naphthylamine.[6,7] As shown in Fig. 4, commercial 1-naphthylamine may include 2-naphthylamine and it is therefore not surprising that 2-naphthylamine is detected along with 1-naphthylamine.

Aromatic amines can be used as diazo components as well as coupling components in the synthesis of azo dyes. When 1-naphthylamine is used as a diazo component, it will be formed after the reduction of the dye. If a dye is made from crude 1-naphthylamine contaminated with 2-naphthylamine, it will also release 2-naphthylamine in addition to 1-naphthylamine under reduction conditions. Examples are the dyes **1** and **2**. As shown in Fig. 5, both 2-naphthylamine and 1-naphthylamine were detected. It is necessary, therefore, that only purified 1-naphthylamine should be employed in the synthesis of azo dyes for commercial use.

Azo dyes synthesized from the use of 1-naphthylamine as coupling component, will release 1,4-diaminonaphthalene after reduction. For example, the reduction of dye **3** gave only 1,4-diaminonaphthalene, as shown in Fig. 6. However, 1-naphthylamine and 2-naphthylamine, besides 1,4-diaminonaphthalene, were detected after reduction of dye **4**, and the peak ratio of 2-naphthylamine vs 1-naphthylamine in Fig. 6 was significantly higher than in Fig. 4. These results imply that 1-naphthylamine contaminated with 2-naphthylamine was used for the synthesis of dye **4** and that the azo-coupling reaction, in which the reaction rate of 2-naphthylamine was slower than that

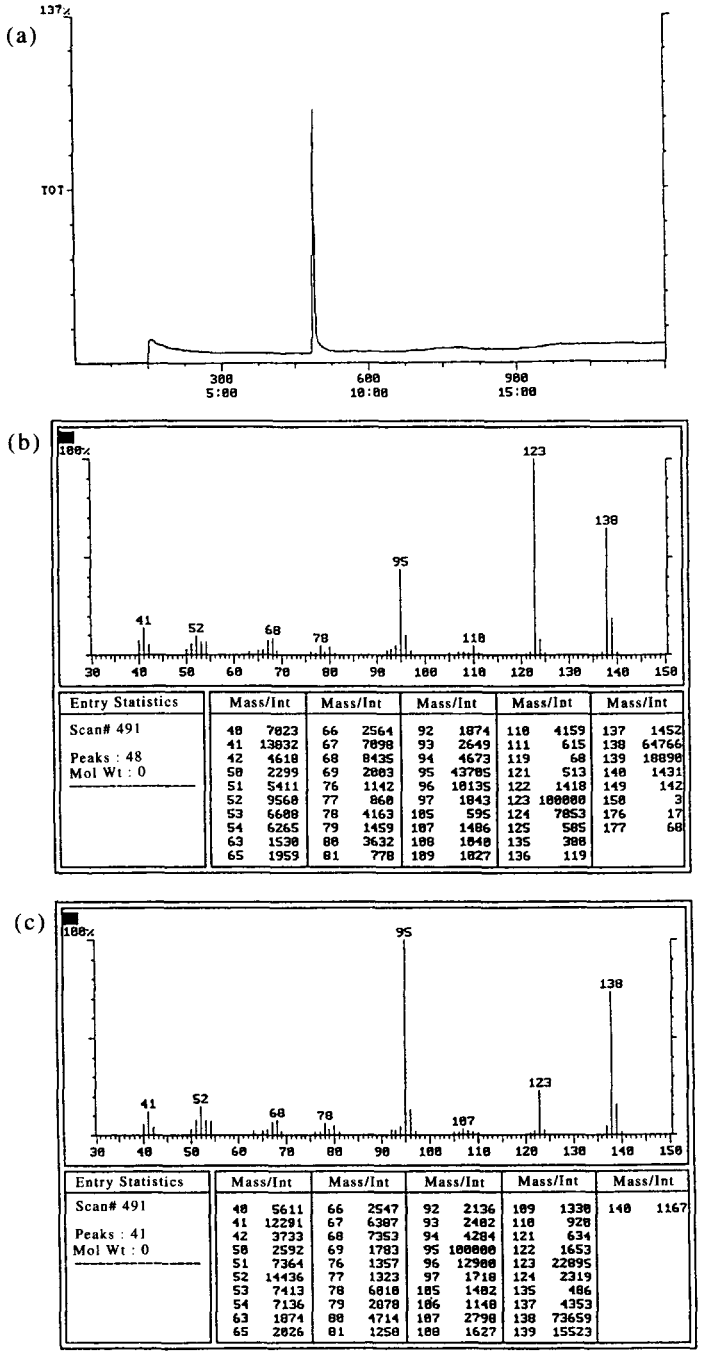


Fig. 1. (a) GC of 2,4-diaminoanisol and 2,5-diaminoanisol. (b) MS of 2,4-diaminoanisol. (c) MS of 2,5-diaminoanisol.

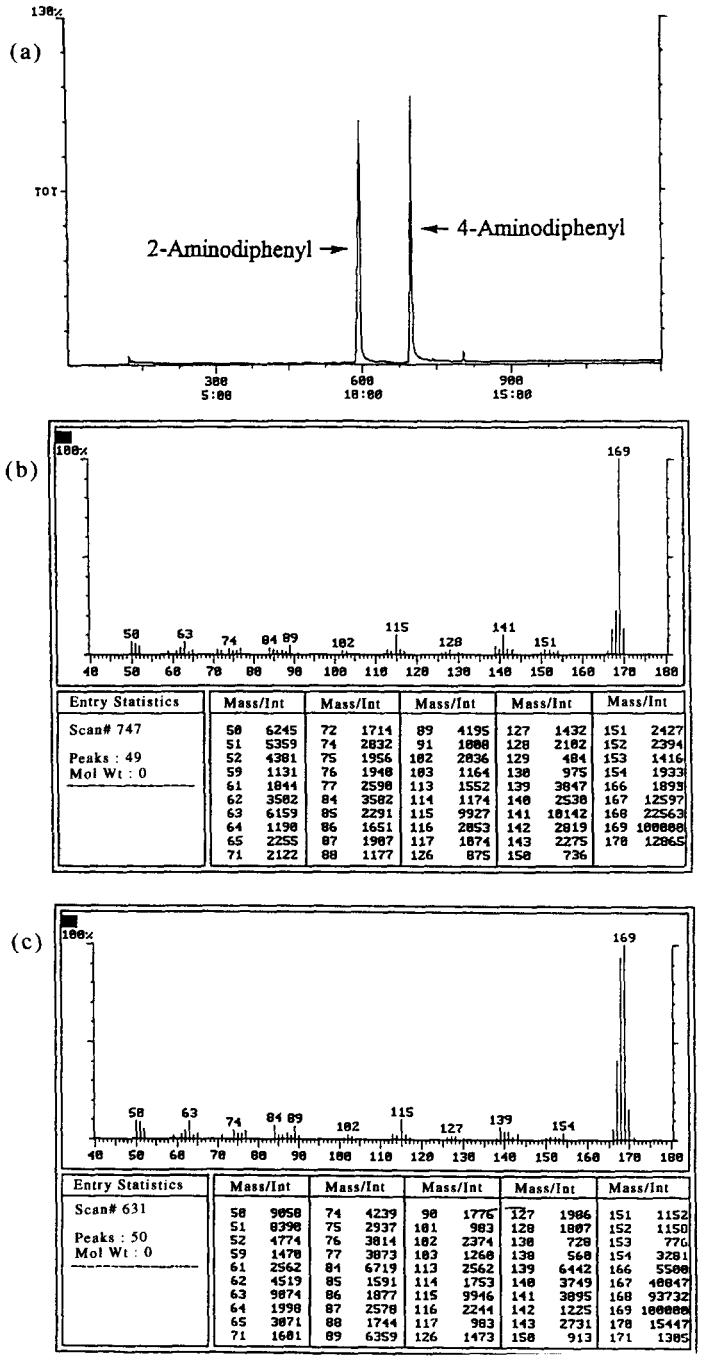


Fig. 2. (a) GC of 2-aminodiphenyl and 4-aminodiphenyl. (b) MS of 4-aminodiphenyl. (c) MS of 2-aminodiphenyl.

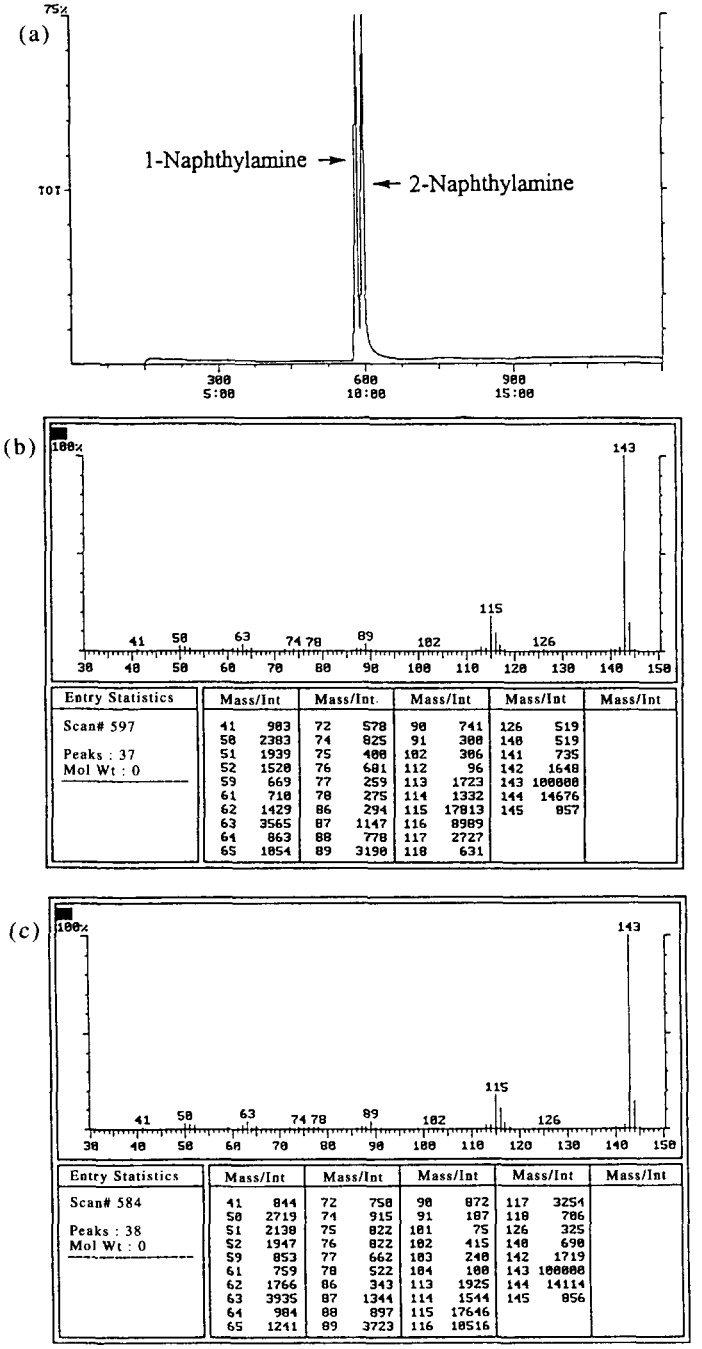


Fig. 3. (a) GC of 1-naphthylamine and 2-naphthylamine. (b) MS of 2-naphthylamine (c) MS of 1-naphthylamine.

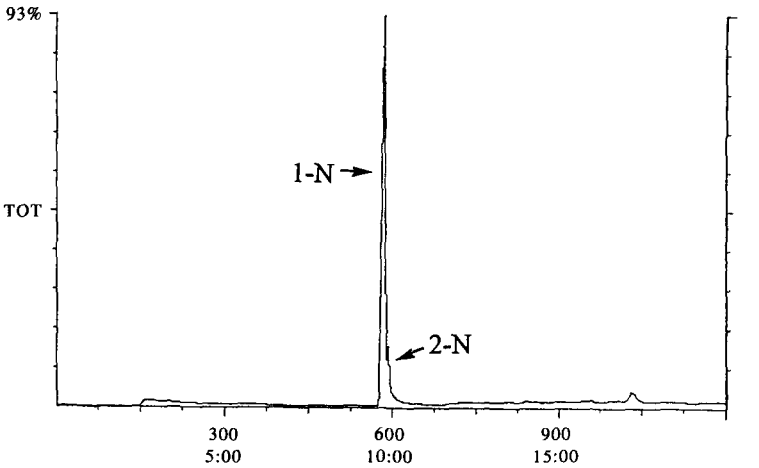


Fig. 4. GC of commercial 1-N.

of 1-naphthylamine, did not go to completion. If 1-naphthylamine contaminated with 2-naphthylamine is employed for the synthesis of dyes, 2-naphthylamine will always be detected because complete reaction is not easy, and moreover the reaction rate of 2-naphthylamine is slower than that of 1-naphthylamine. It is again therefore very evident that the use of purified 1-naphthylamine is required for the synthesis of acceptable azo dyes.

Figure 7 shows examples in which 2-naphthylamine was detected from dyed substrates such as textiles and leather. Although 2-naphthylamine was a minor component, the amount detected from the dyed substrate was above the allowed limit value (50 mg/kg for dyestuff and 5 mg/kg for dyed substrate). Such dyestuffs, which contain trace amounts of the carcinogenic amines, should therefore not be used in the dyeing of consumer goods (or as colour-matching components).

2,4-Toluenediamine and 4,4′-diaminodiphenylmethane

2,4-Toluenediamine and 4,4′-diaminodiphenylmethane were often detected in analysis of materials even though they were not used in synthesis of the azo dyes. Most PU (polyurethane) polymers used for PU foam, PU pigment toners or PU finishing agents in textile and leather are made from 2,4-toluenediisocyanate or 4,4′-diphenylmethanediisocyanate, and the easily accessible moieties in the PU are converted into 2,4-toluenediamine or 4,4′-diaminodiphenylmethane by alkaline hydrolysis without a reduction process. The 2,4-toluenediamine and 4,4′-diaminodiphenylmethane therefore originated from the PU polymers, and not from the colorants.

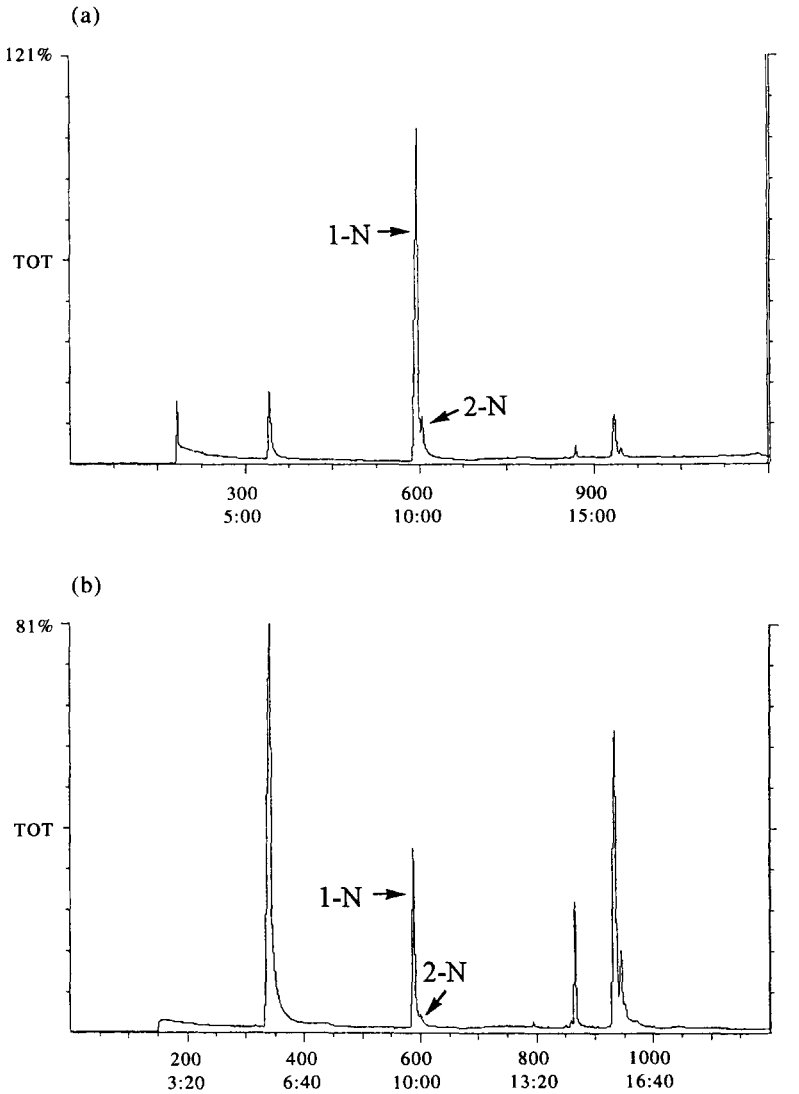


Fig. 5. (a) GC of reduction products of dye **1**. (b) GC of reduction products of dye **2**.

4-Aminodiphenyl

4-Aminodiphenyl is not, to the best of our knowledge, used in the synthesis of commercial azo dyes. However, 4-aminodiphenyl was repeatedly detected as a minor component after reduction of a dye repeatedly during our work. Dye **5** was studied in order to clarify the reason why 4-aminodiphenyl was

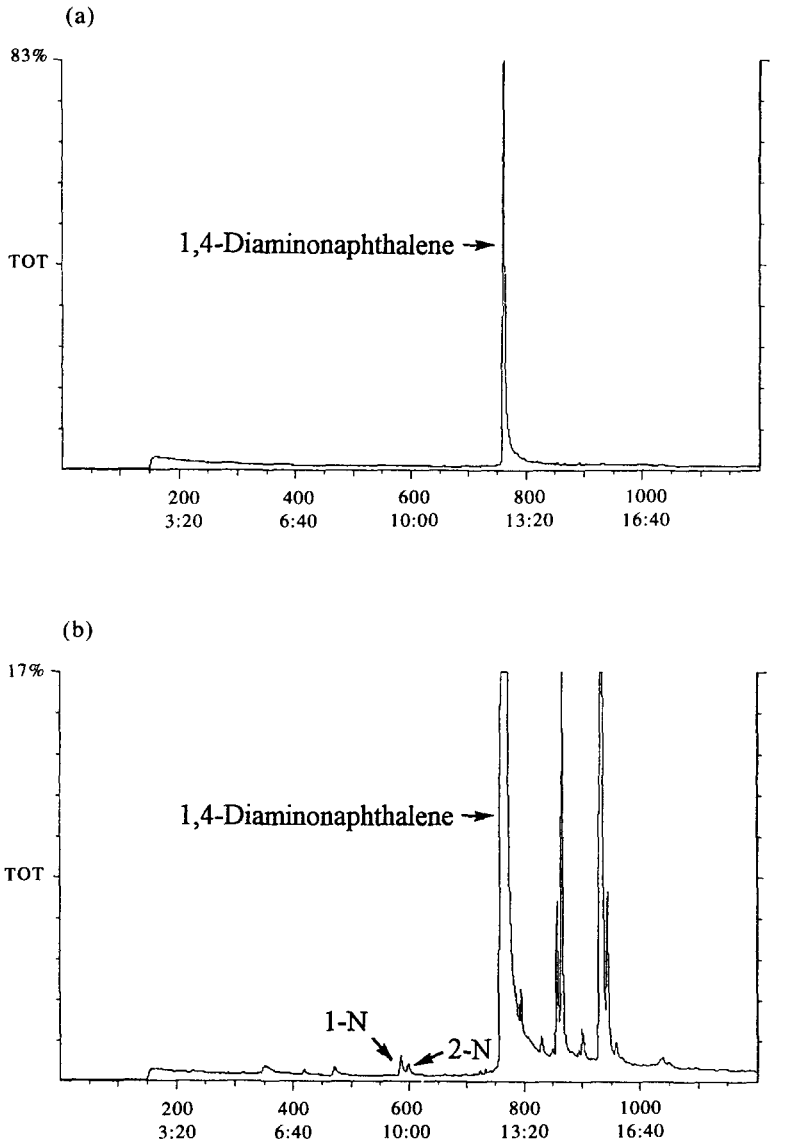


Fig. 6. (a) GC of reduction products of dye **3**. (b) GC of reduction products of dye **4**.

detected. We considered two possible sources for the detection of 4-aminodiphenyl after the reduction of dye **5**. Firstly, 4-aminodiphenyl could arise from its presence as a contaminant in the dye; secondly, 4-aminodiphenyl could be derived from a compound containing a 4-aminodiphenyl moiety. We found that 4-aminodiphenyl was detected after reduction of dye **5**, but not before reduction. From this we concluded that free 4-aminodiphenyl was

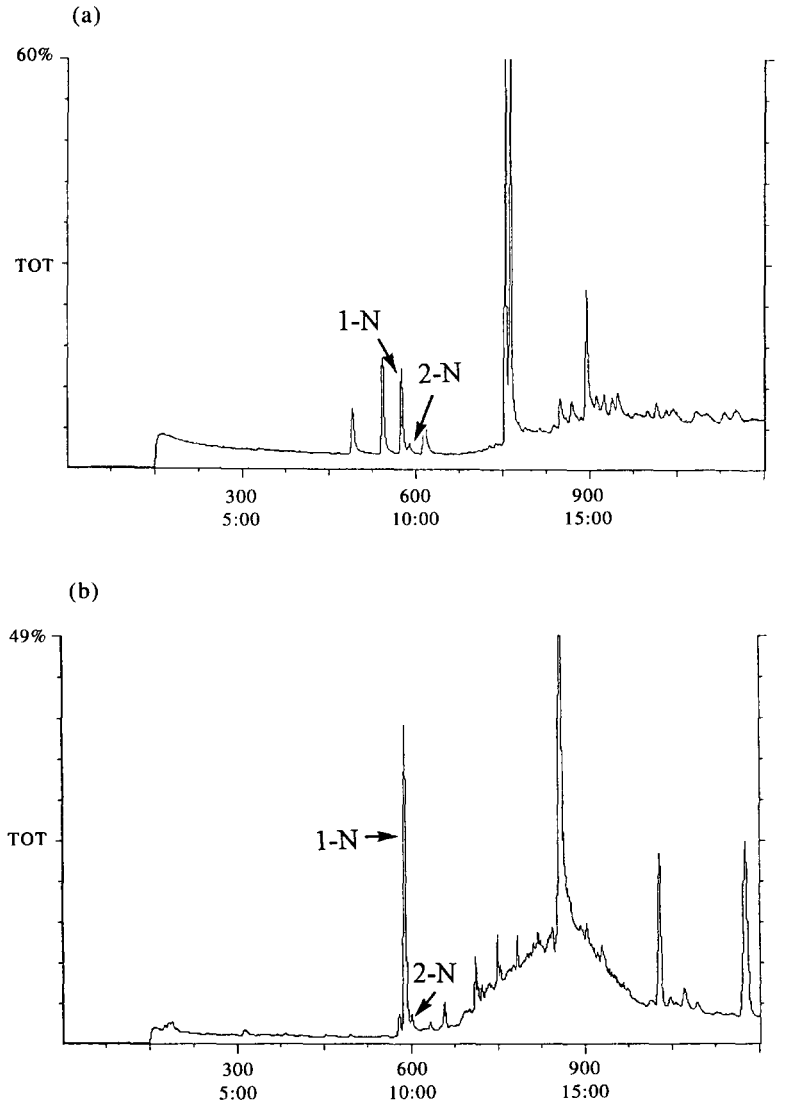
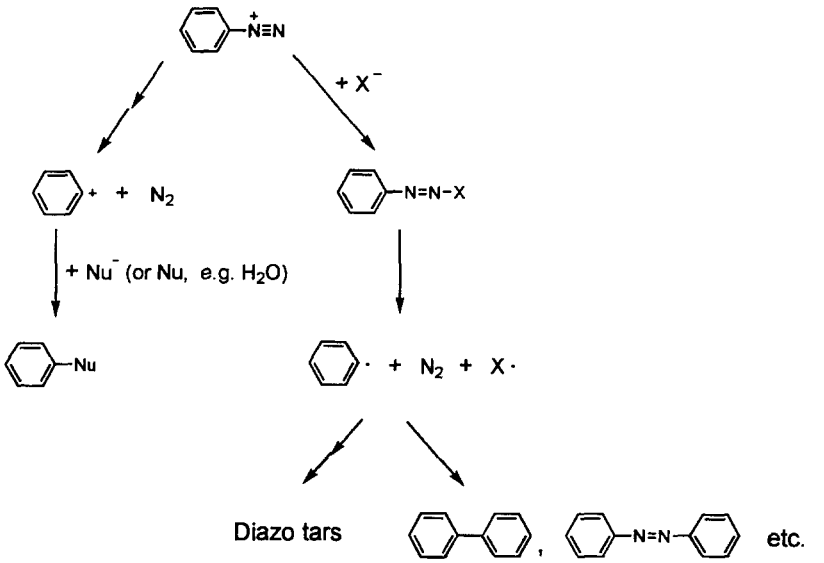


Fig. 7. (a) GC reduction products of dyes extracted from textile. (b) GC of reduction products of dyes extracted from leather.

not present in the dye, but that it arose from a compound containing the 4-aminodiphenyl moiety. The synthetic procedure for dye **5** is shown in Scheme 2. After testing all of the starting materials and products to clear up the source of 4-aminodiphenyl, we found that 4-aminodiphenyl was detected after reduction of compound **B** but not before reduction. From the fact that 4-aminodiphenyl was detected from compound **B**, and not from the

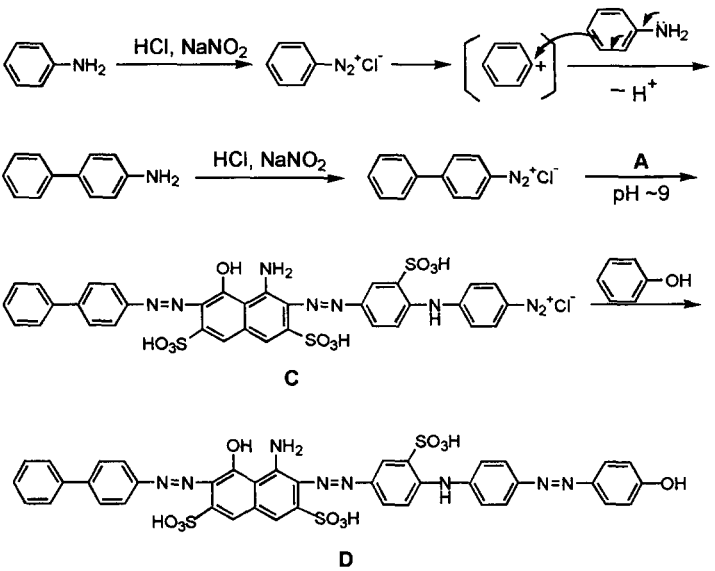


Scheme 3

compound **A** and aniline, we conclude that the source of 4-aminodiphenyl is the diazonium ion of aniline.

It is well known that the aromatic diazonium ion is subject to side reactions during the diazo-coupling reaction, as shown in Scheme 3.[8–10] The C–N bond of the diazonium ion may dissociate heterolytically or homolytically, depending on the structure of the diazonium ion and reaction conditions. In the heterolytic dediazoniation, an aryl cation is formed and this highly reactive species couples easily with any nucleophile. In the presence of a nucleophile X^-, homolytic dediazoniation becomes dominant. The resulting aryl radicals produce biaryl or diazo tars through dimerization or polymerization.

The reason why 4-aminodiphenyl was detected in compound **B** can be explained as shown in Scheme 4. The diazonium ion of aniline is dediazonized heterolytically to give the benzene carbocation and this highly reactive carbocation then undergoes nucleophilic attack by other aniline molecule in the generation step of the diazonium ion, i.e. in acidic conditions, to produce 4-aminodiphenyl. The 4-aminodiphenyl thus obtained was then diazotized to give the diazonium ion of 4-aminodiphenyl, which reacted with **A** to produce **C**. Compound **C** will liberate 4-aminodiphenyl under reductive conditions. It is unlikely that the dediazoniation of the diazonium ion of aniline and successive coupling with unreacted aniline took place in the coupling step, i.e. in alkaline coupling conditions, because free



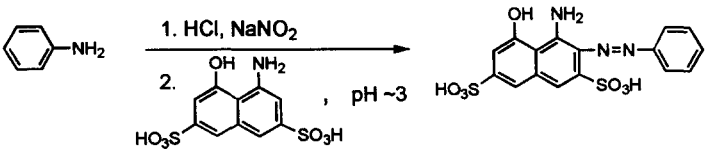
Scheme 4

4-aminodiphenyl was not present either in the intermediate **B** or the dye **5**, as previously noted.

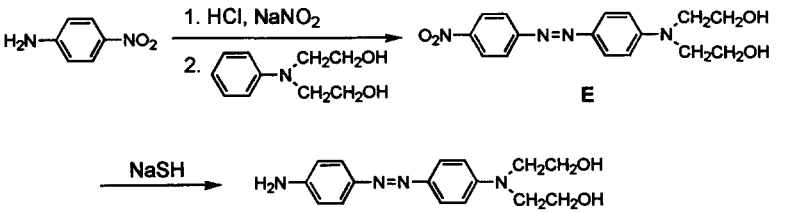
We attempted to couple the diazonium ion of aniline with H-acid in acidic conditions to establish whether 4-aminodiphenyl was formed in the generation step of the diazonium ion of aniline (Scheme 5). It was found that 4-aminodiphenyl was detected from the crude product after reduction but not before. This fact supports the conclusion that 4-aminodiphenyl was already formed and diazotized again in the acidic generation step of the benzene diazonium ion to couple with H-acid. Some literature references[11–15] have reported that the heterolytic dediazoniation reaction of the arene diazonium ion and reaction of the resulting carbocation with a nucleophile occurs under acidic conditions.

Benzidine

C.I. Disperse Black 9 does not contain a benzidine moiety, but it was found that reduction of C.I. Disperse Black 9 produced benzidine. Benzidine was also detected after reduction of the intermediate **E**. We examined the synthetic procedure of C.I. Disperse Black 9 to clarify the reason why benzidine was detected. As shown in Scheme 6, the diazonium ion of 4-nitroaniline is coupled with *N*,*N*-bis-β-hydroxyethylaniline to furnish the diazo compound **E**, which is then reduced to produce C.I. Disperse Black 9. Following the processes discussed in the previous section, any benzidine formed would



Scheme 5



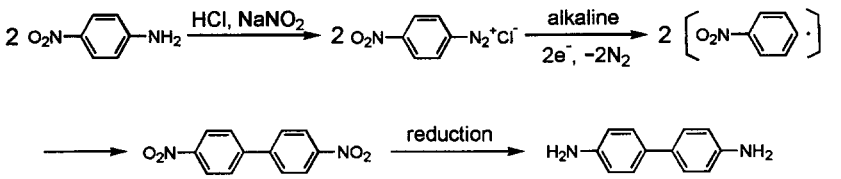
C.I. Disperse Black 9

Scheme 6

have been derived from a diazo component source, and not from a coupling component source. Thus, it was presumed that benzidine was obtained through the dediazoniation of the diazonium ion of 4-nitroaniline, subsequent self-coupling and reduction. As shown in Scheme 7, the diazonium ion of 4-nitroaniline can be dediazonized homolytically to generate the 4-nitrophenyl radical, evolving nitrogen gas. The homolytic dediazoniation would occur in the coupling step, i.e. in alkaline conditions. The Gomberg–Bachmann reaction,[16–21] which is a method for the arylation of aromatic compounds, takes place through homolytic dediazoniation of aromatic diazonium ions and subsequent coupling with aromatic compounds in aq. alkaline solution. The resulting 4-nitrophenyl radicals would self-couple to give 4,4′-dinitrobiphenyl, which can then produce benzidine after reduction. Under this presumption we examined compound **E** and succeeded in isolating 4,4′-dinitrobiphenyl from **E** before reduction. This confirms that the C.I. Disperse Black 9 sample used was contaminated with free benzidine resulting from the reduction of 4,4′-dinitrobiphenyl.

CONCLUSION

'German Regulations on Consumer Goods' did not define the allowed limit in the contents of the 20 carcinogenic amines. However, ECO labels are given to daily goods containing only tiny amounts of the 20 harmful amines,



Scheme 7

generally less than 50 mg/kg for dyestuff and less than 5 mg/kg for dyed substrate. Our investigations frequently found that the detected quantities of 2-naphthylamine, 4-aminodiphenyl and benzidine (in the cases specifically discussed above), amounted to more than the allowed limit. Therefore, even though harmful amines are not employed in the manufacture of a dyestuff, it is still necessary to confirm whether they are detectable from the dyestuff or not, and dyestuff showing their presence should not be used in dyeing consumer goods. When harmful amines not used in manufacture of dyestuffs are liberated, it is also desirable to ascertain their origin, and reaction conditions which do not produce these harmful amines should be studied for use in the dye synthesis. Where such methods cannot be developed, precursors which can yield harmful amines should be replaced by other intermediates.

2,4-Toluenediamine and/or 4,4′-diaminodiphenylmethane were detected from almost all the substrates containing PU foam or PU finishing agent. Although 'German Regulations on Consumer Goods' did not state prohibition for the harmful amines detected from substrates other than dyestuffs, it should be considered whether the 2,4-toluenediisocyanate and 4,4′-diphenylmethanediisocyanate moieties used for the manufacture of PU should be substituted by other systems which inhibit the formation of 2,4-toluenediamine and 4,4′-diaminodiphenylmethane. It must be noted that 2,4-toluenediamine and 4,4′-diaminodiphenylmethane are released only by hydrolysing extracts of PU foams and PU finished substrates, without any reduction conditions. Therefore, PU containing the 2,4-toluenediisocyanate or 4,4′-diphenylmethanediisocyanate moiety may therefore be potentially more dangerous than harmful azo dyestuffs.

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